MULE DEER (*ODOCOILEUS HEMIONUS*) REPRODUCTION, FAWN SURVIVAL, AND EXPOSURE OF FAWNS TO INFECTIOUS AGENTS IN A CHRONIC WASTING DISEASE ENDEMIC AREA OF SOUTHERN SASKATCHEWAN

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By

RANAWAKA ARACHCHIGE ASHA PRIYASHANTHI PERERA

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ABSTRACT

As a part of an ongoing study of the geographic spread and long term population effects of chronic wasting disease (CWD) in southwestern Saskatchewan, we captured and radio-collared female mule deer (*Odocoileus hemionus*) in the winter of 2009, 2010 and 2011 and, in the subsequent spring, we captured their newborn fawns. Fawns were blood sampled, radio-collared and ear tagged, released and then continuously monitored for survival status until the following February or March. During the winter capture we also sampled blood, tonsils and feces from juvenile mule deer which were approximately 8 months old. Our primary objectives were to 1) estimate female reproductive indices and evaluate fawn survival rates within the first 8 months of life and 2) detect exposure to selected infectious agents in neonates and 8-month old juveniles.

Pregnancy rate, as determined by ultrasound in 2010 and 2011 winter was 100% (n=84); the overall average number of fetuses per doe was 1.99 ± SD 0.33 (167 fetuses, 84 does) with a predominance of twins. Mean birth rate was 1.29 ± SD 0.72 fawns per doe (139 neonates, 108 does). During the 3 capture years, we captured and radio-collared 118 neonates (n=38 in 2009, n=41 in 2010, n=39 in 2011). Probability of fawn survival was 0.334 ± SD 0.047 for the first 8 months of life from 2009 to 2011 and did not differ among capture years (P = 0.411). The most common known cause of mortality was predation. Having a longer body length at birth reduced the risk of fawn death for 0 - 7 day postpartum period. Members of larger litters had a higher risk of death during 8 – 30 day postpartum period. However, subclinical CWD status of dam was a poor predictor of fawn survival during the first 8 months of life.

We performed various laboratory tests on feces, blood and tonsils of 8-month-old deer to detect prevalence of parasitic agents shed in feces, CWD in tonsils, mule deer lymphotropic herpes virus (mule deer-LHV) in buffy coat and presence of antibody titers in serum to bovine

viral diarrhoea virus (BVDV), bovine herpes virus-1 (BHV-1), parainfluenza 3 virus (PI3), and the protozoan parasite *Neospora caninum*. Fecal analysis revealed a high prevalence for *Orthostrongylus* sp.: 86% (80/93), and moderate prevalence for *Moniezia*: 29% (27/93) and *Thysanasoma*: 29% (27/93). Seroprevalence for BHV-1, BVDV, PI3 virus and *Neospora caninum* was 3% (3/92), 20% (19/93), 22% (20/93), and 3% (3/91) respectively. Prevalence of mule deer-LHV was 15% (13/85). In 2010 and 2011, we detected subclinical CWD infection in 2 and 1 juveniles, respectively, with an overall prevalence of 3% (3/95). Serology on neonate blood revealed 12% (13/106) were seropositive for *N. caninum* and all dams that were seropositive for *Neospora* gave birth to at least one fawn.

Mule deer in the Antelope Creek area of southwestern Saskatchewan have low fawn survival rates which may, in combination with high adult mortality due to hunting or CWD, result in population declines. Given the long incubation of CWD, even a low prevalence of CWD in 8 month old fawns suggests high infection pressure on resident deer. The high prevalence of gastrointestinal parasites and moderate seroprevalence for BVDV and PI3 virus at 8 months of age indicates frequent exposure to pathogens spread via the environment and by direct transmission. Because these are routes of transmission shared by CWD, this moderate to high prevalence of exposure to infectious agents also supports the hypothesis that fawns are potentially exposed to CWD at a young age. To understand factors responsible for the observed annual variability in exposure to *Orthostrongylus*, BVDV, and PI3 virus requires investigation of these infectious agents in sympatric species including livestock in the area.

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DEDICATION

To my parents, brother, sister and Kamal for their unending love and care…

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIC	Akaike's Information Criterion
ANOVA	Analysis of Variance
BHV	Bovine Herpes Virus
BM	Body Mass
BRI	Body Reserve Index
BVDV	Bovine Viral Diarrhea Virus
°C	Centigrade
CI	Confidence Interval
cm	Centimeters
CWD	Chronic Wasting Disease
df	Degrees of Freedom
DPOL	DNA Polymerase
ELISA	Enzyme-Linked Immunosorbent Assay
FAT	Fluorescent Antibody Test
GPS	Global Positioning System
g	Gram
IFBF %	Ingesta free body fat percentage
kg	Kilogram
LHV	Lymphotropic Herpes Virus
MAXFAT	Maximum Fat Thickness

OD_{NC}	Optical Density of Negative Control
PCR	Polymerase Chain Reaction
PET	Precise Event Transmitter
PI3	Parainfluenza 3 Virus
PrP	Prion Protein
rBCS	Rump Body Condition Score
RuRV	Ruminant Rhadinovirus
SD	Standard Deviation
SE	Standard Error
SNT	Serum Neutralization Test
TCID	Tissue Culture Infective Dose
TSE	Transmissible Spongiform Encephalopathy
VHF	Very High Frequency
VIT	Vaginal Implant Transmitter
WSI	Winter Severity Index

CHAPTER 1

LITERATURE REVIEW

1.1 Chronic Wasting Disease

Chronic wasting disease (CWD) is a prion disease of North American cervids detected in 18 states of the United States and in 2 Canadian provinces (Figure 1-1). Chronic wasting disease affects members of the family Cervidae: mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus),* black-tailed deer (*Odocoileus columbianus*), Rocky mountain elk (*Cervus elaphus*) and moose (*Alces alces shirasi*) (Sigurdson 2008). Experimental oral infection has been reported recently in reindeer (*Rangifer tarandus tarandus*) (Mitchell et al. 2012). Though first reported in Colorado in 1980, it had been recognized as a wasting syndrome of captive deer for many years before (Williams and Young 1980). The causative agent is an altered version of a cellular glycoprotein called prion (PrP^c) which is present in normal living cell membranes. Although what exactly triggers the misfolding is under debate, once a PrP^c molecule has changed its morphology, it can act as a template for a chain of aberrant folding of other PrP^c and accumulation, causing cellular and tissue changes and, ultimately, fatal clinical disease (Prusiner 2004). Clinically, the predominant signs of CWD at its initial stages are progressive weight loss, emaciation and behavioral changes, while in latter stages of disease, salivation, ataxia, lowered head and drooped ears, fixed stare, lack of awareness, hyperexcitability and rough hair coat may be displayed (Williams 2005). The mode of transmission is via ingestion or inhalation of misfolded prion protein that is shed in feces, saliva, urine or persistence after decomposition of an animal dead of CWD. Ingestion occurs primarily through animal to animal contact or via contaminated environments; vertical transmission has been documented but is thought to be relatively unimportant (Miller and Williams 2003). After infection, time to initial clinical signs can vary from weeks to years because of the characteristic

slow progression of CWD. Clinical disease is rare in yearling deer (Williams 2005). The disease is invariably fatal.

Other diseases caused by abnormal prions include bovine spongiform encephalopathy (BSE) which affects cattle, scrapie in sheep and goats, transmissible mink encephalopathy and Creutzfeldt- Jacob disease in humans. These diseases, as well as CWD, are classified as transmissible spongiform encephalopathies (TSEs), because of the characteristic histopathological finding: formation of intracytoplasmic vacuoles in neurons and neuronal processes, which give the central nervous tissue a sponge-like appearance on histological examination (Williams 2005). Zoonotic transmission of chronic wasting disease has not been reported (Saunders et al. 2012).

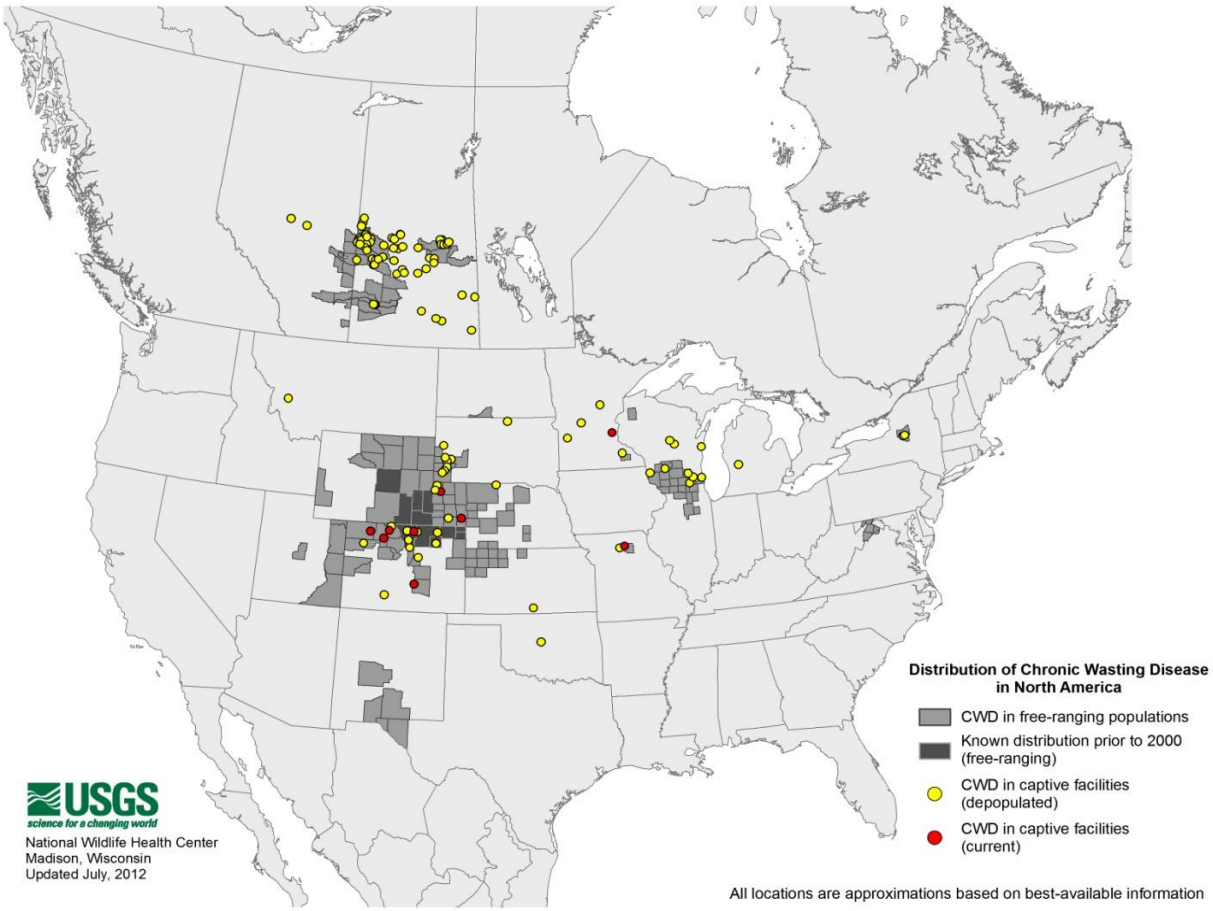

Figure 1-1. Current distribution of chronic wasting disease in North America. http://www.nwhc.usgs.gov/images/cwd/cwd_map.jpg. Accessed on 15 July 2012.

1.1.1 Chronic wasting disease in Saskatchewan

Chronic wasting disease was first diagnosed in Canada in 1996 in a farmed elk near Regina, Saskatchewan. Four years later, CWD was confirmed in a hunter-shot wild mule deer in the west central part of the province (Bollinger et al. 2004). Evidence indicates CWD was introduced into Saskatchewan in the 1980s through the importation of infected farmed elk from the USA and then it subsequently spread to wild cervids (Kahn et al. 2004). Since then, CWD has continued to spread throughout the province and multiple cases have been identified in mule deer, white-tailed deer and elk. Cases have not yet been detected in moose in Saskatchewan (Bollinger et al. 2009). In some wildlife management zones, the prevalence is currently approximately 5%, while in smaller areas such as our Antelope Creek study area in southern Saskatchewan, the prevalence is greater than 25% in wild mule deer (Bollinger 2011) (Figure 1-2).

Immediately after CWD was discovered in farmed elk, surveillance programs were established using hunter-submitted deer heads in order to determine the geographic extent of the disease and to monitor its spread. When new cases were identified, herd reduction programs were implemented in the newly affected region to attempt to prevent further spread. Incentives such as earn-a-buck hunting permits (deer hunting permit to hunt a male or female deer that is issued after a hunter submits two female/fawn mule deer) were issued to hunters who submit deer heads for surveillance in order to encourage sample submission. Mobile sample collection stations which receive deer heads from hunters are currently used to increase the number of samples tested from areas of Saskatchewan where CWD has not been detected but sample sizes are still low (Figure 1-2) (Saskatchewan Ministry of Environment 2011a).

In the Unites States, CWD in combination with predation by mountain lions (*Puma concolor*) has reduced the abundance of free-ranging mule deer (Miller et al. 2008). The effects

of CWD on Saskatchewan wild deer populations and its long term ecological impacts are unclear.

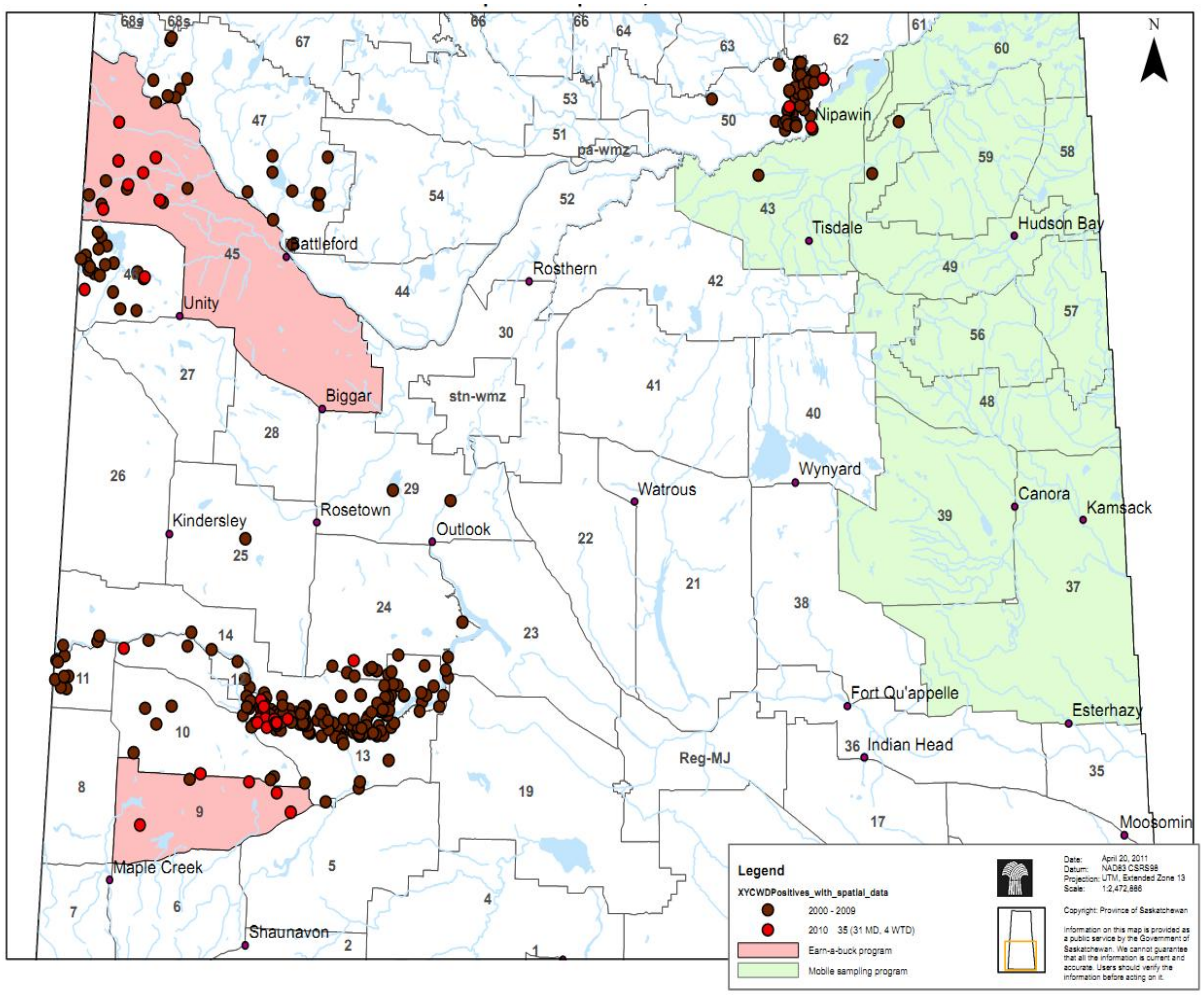

Figure 1-2. Distribution of chronic wasting disease cases found in Saskatchewan, Canada in 2000-2010, and location of enhanced sample collection programs: Red circles – locations of chronic wasting disease cases in 2010, Brown circles – locations of chronic wasting disease cases from 2000-2009, pink shaded area - Earn a buck program in 2011, green shaded area - mobile sample collection program in 2011, small circles are towns (Saskatchewan Ministry of Environment 2011b).

1.1.2 CWD Management Strategies

Various strategies have been employed to try to eradicate CWD from captive facilities and free-ranging deer environments. Culling of affected herds and all in-contact animals, quarantine, continuous surveillance, cleaning and disinfection have been performed in infected captive facilities (Miller et al. 1998) while herd reduction is the primary method of management that has been attempted in wild cervid populations (Argue et al. 2007). In spite of all attempts to manage the disease, CWD continues to spread geographically in the United States and Canada and the prevalence continues to increase in many areas.

The effectiveness of various CWD control measures is difficult to assess due to long incubation periods which can span months to years; indirect transmission mainly through contaminated environments; persistence of prions in the environment that lasts several years and the resistance of prions to most available disinfectants. Because it currently is not possible to determine the rate and intensity of environmental contamination during subclinical and clinical stages of CWD in free-ranging or captive deer herds, efficacy of herd reduction as a control measure remains unknown. Long distance movements of juvenile deer of more than 100 km, limit the effectiveness of current CWD management zones which are typically smaller than deer dispersal distances (Skelton 2010). If the persistence of infectious prions in the environment is long and if indirect transmission plays a major role in CWD transmission, the environmental reservoir of CWD prions will increase progressively (Almberg et al. 2011); and therefore, much higher prevalence rates and more severe population effects can be expected. The same authors also suggested that if infected individuals could be diagnosed at an early stage of incubation using improved diagnostic tools, targeted culling may be an effective mode of disease control by limiting growth of the environmental reservoir. However; the technology for such early detection of CWD does not now exist.

In order to better control the spread of CWD, further research is required to fill knowledge gaps on possible CWD strains, host population effects, prion detection tools for soil and other environmental substrates, the role of direct and indirect transmission, prion persistence in environment and means of effective disinfection of contaminated environments.

1.2 Mule Deer (*Odocoileus hemionus*)

Mule deer are small bodied wild ruminants distributed throughout most of western North America, ranging from northern Mexico to the southern Yukon, Canada. They are an important game species and are popular with the general public because they are common and easily observed (Kie and Czech 2000). Mule deer play a key role in maintaining health of natural ecosystems by having substantial influences on vegetation (Hanley 1996, Hobbs 1996).

Taxonomically, mule deer are in the Order Artiodactyla, the even-toed, hoofed mammals, Suborder Ruminantia, Family Cervidae, and Genus *Odocoileus* which also includes their evolutionary cousin, the white-tailed deer. Black-tailed deer are the same species as mule deer (Anderson and Wallmo 1984, Mackie et al. 2003). The Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) is the subspecies with the widest range and is the subspecies found in Saskatchewan (Kie and Czech 2000). The maximum life span of mule deer is about 20 years (Ross 1934). However; in the wild, longevity does not often exceed 10-12 years for females and 8 years for males (Kie and Czech 2000). Adult male body mass ranges from 90 to 115 kg whereas females reach their maximum body mass of 80 kilograms by their second year of life when they often give birth for the first time (Anderson et al. 1974).

1.2.1 Social Structure

Adult mule deer frequently form female groups consisting of females of several breeding years and their offspring which often share a maternal lineage. Males usually leave these female groups as juveniles and form cliques of unrelated individuals (Geist 1981). Throughout most of

the year, adult males and females do not associate, except for late fall during the mating season (Bowyer 1984). In populations of mule deer protected from hunting, the adult female to male ratio is often more than 2:1 (Mackie et al. 2003).

1.2.2 Habitat

Rocky mountain mule deer live in diverse habitats including short grass and shrub lands of the northern prairies, woodlands and forest areas of Rocky Mountain regions and hot and arid southern desert lands. On Montana prairies, summer foods of mule deer consisted of forbs, mainly yellow sweetclover (*Melilotus officinalis*), and shrubs including silver sage brush (*Artemisia cana*), and thorny buffalo-berry (*Shepherdia argentea*), while grass was only utilized in minute quantities (Dusek 1975). The same author further found that snowberry (*Symphoricarpos* spp.) and rubber rabbitbrush (*Chrysothamnus nauseosus*) were commonly consumed in the fall, while winter diet included rubber rabbitbrush (*Chrysothamnus nauseosus*) that increased in proportion in diet with progression of winter, Rocky Mountain juniper (*Juniperus scopulorum*), creeping juniper (*Juniperus horizontalis*), and soapweed (*Yucca glauca*).

1.3 Female Reproduction

Male mule deer mate with multiple females in a single mating season (Geist 1981). Most females first breed in the second fall of their life when they reach 1.5 years of age. The duration of the single annual oestrus cycle of an adult doe can range between 22 to 28 days. During oestrus, the receptive period lasts for 24 to 36 hours (Mackie et al. 2003). In mule deer populations, the pregnancy rate can range from 70% to > 90%, varying with nutritional status, weather conditions and population density (Mackie et al. 2003). The average pregnancy period is 203 days, but can range from 188 to 218 days (Robinette et al. 1973). Females give birth in spring when conditions are favourable for survival of a new born. During the last few weeks of

gestation and the first 3-4 weeks after parturition, females exhibit territoriality towards their own juveniles as well as other deer. Neonatal fawns remain hidden during most hours of the day during the first 6 to 8 weeks of life (Geist 1981).

1.3.1 Age

Pregnancy in the first year is rare in wild mule deer compared to white-tailed deer (McCullough 1997). However; mule deer fawns that were fed a highly nutritious diet became pregnant during their first fall in a captive facility in Colorado (Robinette et al. 1973).

The rate of pregnancy is lower in yearling mule deer does than in older females. Reproductive capacity of yearling females may differ annually according to weather conditions, forage availability and population density, whereas the fecundity of older females is less sensitive to, and more independent of, most environmental factors (Gaillard et al. 2000). Primiparous females between 1-2 years of age tend to carry fewer fetuses than older breeding females, which may bear up to 1.7 to 2.25 fetuses per doe (Jensen and Robinette 1955, Andelt et al. 2004, Tatman 2009). Females carrying triplets and females which fail to breed are uncommon in mule deer (Robinette et al. 1955).

1.3.2 Body Condition

Fat, which is stored as triglycerides in the body, is a key source of stored energy, and fat reserves are the main indicator of nutritional condition of an ungulate. Subcutaneous fat stores are depleted first and marrow fat is depleted last, while kidney fat depletion occurs in the middle of the utilization sequence (Cederlund et al. 1989).

Adult female fat reserves are a major factor governing the reproductive performance of ungulates (Testa and Adams 1998, Keech et al. 2000). Ovulation failure (Tanaka et al. 2003), implantation failure (Sosa et al. 2004), low pregnancy rate (Mani et al. 1996, Tanaka et al. 2003)

and reduced birth rate (Russell et al. 1998, Cook et al. 2004) can be unfavorable reproductive consequences of impaired nutritional status in female ruminants.

Numerous methods have been proposed to assess nutritional condition of both live and dead ungulates. Common live animal condition assessment methods include body condition scoring by palpation (Gerhart et al. 1996a), measurement of subcutaneous rump fat and muscle thickness using ultrasound scanning (Stephenson et al. 1998), body mass (Jiang and Hudson 1994), serum and urine chemistry (Kirkpatrick et al. 1975, Cook et al. 2001) and bioelectrical impedance analysis (Cook 2000, Hundertmark and Schwartz 2002). Femur marrow fat (Mech and Delgiudice 1985), mandible marrow fat (Davis et al. 1987), metatarsal marrow fat (Davis et al. 1987), carcass-scoring techniques such as Kistner scoring (Kistner et al. 1980), kidney fat (Finger et al. 1981, Anderson et al. 1990) and water dilution (Torbit et al. 1985) are indices used in evaluation of condition in dead animals.

Recently, several of these techniques which primarily estimate body fat deposits have been validated for mule deer (Stephenson et al. 2002, Cook et al. 2007). However; the range of body conditions over which each index is useful differs. Body condition score, Kistner score and rLIVINDEX (an arithmetic combination of rump subcutaneous fat thickness and body condition score) can be used for condition evaluation across the total range of conditions from poor to good condition. Marrow fat is only useful in deer in poor condition, while subcutaneous fat thickness and Wyoming index are used in deer in moderate and high nutritional condition (Cook et al. 2007).

1.3.3 Winter Weather

Winter weather is a principal determinant of body condition and survival of deer of northern latitudes (Runge and Wobeser 1975). During cold months of the year ungulates consume poor quality forage which is scarce and hard to find (Short et al. 1966). Deep snow,

crusted and ice-covered snow and prolonged snow cover can restrict access to low-lying or ground level food sources such as native grasses, forbs and agricultural waste cereal crops which are important sources of energy for deer. Deep snow depth can make movements difficult and energetically costly for wintering deer (Runge and Wobeser 1975, Farnes 2002). Even in mild winters, deer struggle to maintain their body fat reserves because of decreased abundance of food and the low quality of feed available at this time.

Mean number of fetuses per doe was lower in pregnant white-tailed deer fawns in severe winters compared to mild winters in Saskatchewan, although yearling and adult reproductive rates were not affected (Brewster 1993). Inconsistencies in fetal growth was common in gravid white-tailed females in poor nutritional condition and the degree of fetal size discrepancy increased with increasing litter size (Brewster 1993). Unusually severe cold weather in 1973-74 in Saskatchewan caused a severe decline in body condition in white-tailed deer and lead to loss of some or all fetuses in gravid females (Runge and Wobeser 1975).

1.4 Fawn survival

Survival within the first year of life is often a major element driving deer population dynamics (Gaillard et al. 1998). In some studies, mule deer fawn survival was reported < 30% : 28% in eastern California (Bleich et al. 2006) and 16% for desert mule deer in central Arizona (Tatman 2009) and it changed significantly between years and within and among populations (Bishop et al. 2005, Bleich et al. 2006). Other authors have found high fawn survival: 52% for 108-day summer period (Johnstone-Yellin et al. 2009) and 72% for 6-12 month old fawns in Colorado (Lukacs et al. 2009).

Mule deer normally wean around 4-6 months of age and fawn survival can be calculated for preweaning and postweaning time periods (Anderson and Wallmo 1984, Gaillard et al. 2000). Preweaning survival is lowest within the first 4 weeks after birth (Pojar and Bowden 2004) and is

dependent on a number of factors including late gestational nutritional status of doe, birth weight, maternal reproductive experience and predator pressure in the environment. In contrast, in temperate northern climates, postweaning ungulate survival, until the end of the first winter, is mostly dependent on weather and less affected by dam-related components; mortality is commonly a result of starvation (Gaillard et al. 2000).

1.4.1 Proximate factors affecting fawn survival

Similar to other ungulates, mule deer use a strategy whereby fawns do not follow the dam but remain motionless and hidden during the first two months of life to reduce encounters with predators. Neonates have cryptic coloration with white spots that resemble sunny patches in the vegetation. Dams contribute by ingestion and removal of placenta, moving of fawns from the birth site and placing littermates away from each other and by only making few short visits during a day to nurse neonates (Geist 1981). Despite these behavioural strategies, predation remains the most common cause of death of fawns during the early vulnerable stage in predator rich environments. Predation has been reported to account for between 56 % to 100 % of neonatal mule deer deaths (Linnell et al. 1995). Wolves, bears, bobcats and red foxes accounted for 86% of total mortality of new-born deer in Minnesota (Carstensen et al. 2009). In a sympatric population of mule and white tailed deer in Colorado, 79% of fawn deaths were related to coyote predation (Whittaker and Lindzey 1999) , but in another study in Colorado (Pojar and Bowden 2004), predation accounted for as few as 20 % of total fawn deaths. In the latter study, predator related mortality was the second most common cause of death.

Other proximate causes of pre-weaning and postweaning fawn death include starvation/malnutrition, disease, and trauma/accidents. Sickness and starvation explained most of mule deer fawn mortality during one study year in west-central Colorado (Pojar and Bowden 2004). Lomas and Bender (2007) found that most fawn deaths were caused by malnutrition in

north-central New Mexico and the annual mortality rates ranged between 9% and 32%. Long term survival investigations in Colorado, Idaho and Montana revealed that rates of fawn mortality due to malnutrition during winter varied considerably and differed among years and locations (Unsworth et al. 1999). Starvation and disease can weaken animals and predispose individuals to predation. In a study of proximate causes of fawn death, malnutrition accounted for 20 % of deaths, but most fawns that were dead of predation were in poor condition (Bishop et al. 2009) . In the neonatal stage, malnutrition can be caused by abandonment due to natural causes or because of handling or radio-making activities inducing abandonment.

Deaths as a result of infections are not commonly detected in neonatal deer, but disease should be considered as a possible cause of mortality during this early life stage. Bovine viral diarrhoea virus was isolated from two dead mule deer fawns (Pojar and Bowden 2004), and fawn deaths reported between 2 and 8 weeks of age were due to necrotic stomatitis in a captive facility (Robinette et al. 1973). Myers (2001) documented young mule deer fawns dying of pneumonia and enteric disease, including haemorrhagic enteritis and colitis.

Trauma or accidents may be a minor cause of neonatal death (Pojar and Bowden 2004, Tatman 2009). The type of trauma or accident may vary widely according to landscape and terrain though it often includes physical injury, drowning or being entangled in fences or cactus.

Unknown-fates category is commonly included in ungulate neonatal mortality studies (Pojar and Bowden 2004, Carstensen et al. 2009). This reflects the difficulty of detecting a mortality signal from motion-sensing radio-collars from fawn carcasses that are being frequently moved by predators or scavengers. As well, it is difficult to find carcasses before they are scavenged or become too autolysed for detailed necropsy evaluation.

1.4.2 Other factors affecting fawn survival

Survival of fawns during the preweaning period depends to a large extent on the dam. Weight and physical condition of a neonate at birth are determined by the nutritional status of the doe before and during the pregnancy period. Fawns born with higher weights live longer than low-birth weight neonates (Bishop et al. 2009). Fawns born to prime aged and older females tend to have higher birth weights than younger parturient does (Jose et al. 2000). Spring and summer maternal diets have direct effects on lactation and nutrition of the new born deer. Fawn rearing success was dependent on age of the doe in white-tailed deer while primiparous mothers were not as successful as prime aged, multiparous deer in Michigan (Ozoga and Verme 1986). Experienced does are better at detecting and distracting predators, signalling danger to fawns, defending against predators and moving fawns away from danger than are primiparous females. Older females commonly give birth earlier (Jose et al. 2000, Bishop et al. 2007) and fawns born early in spring experience higher survival than those born later (Bishop et al. 2009).

Litter size and sex of newborn ungulates also may contribute to birth characteristics, as well as to postnatal fawn survival in mule deer. Robinette et al. (1973) found that twin litter mates as well as female litter mates weighed less at birth than singletons and male siblings, respectively. Male neonates tend to have lower survival than female fawns within the first month in some environments (Bishop et al. 2009, Johnstone-Yellin et al. 2009). This is thought to be because of the higher growth rate of males which requires higher food intake and to fulfill these needs, males tend to move more and this makes them more vulnerable to predation. Singletons tend to live longer than twins (Johnstone-Yellin et al. 2009).

1.5 Infectious agents

Mule deer are susceptible to numerous infectious agents, even though the agents may not necessarily be pathogenic and it is well known that mule deer and other wild ungulates share many infectious agents with cattle (Dunn 1969).

Furthermore, young animals either domesticated or wild, tend to be more susceptible to certain infectious agents. For example, fawns had a 100% prevalence of *Haemonchus* spp., while adult deer had an 80% prevalence and a five times lower maximum individual worm burden (Forrester 1992). Low immunological responsiveness in young ruminants compared to mature animals is probably the result of maternal antibodies, insufficient exposure to pathogens to develop active immunity, and incompetent immunological mechanisms in both humoral and cell-mediated responses (Colditz et al. 1996).

1.5.1 Parasitic agents

Large mammals are typically infected with numerous internal as well as external parasites. While most parasites are not harmful to the host, some can be opportunistic pathogens and still others are always pathogenic. A wide variety of parasites have been discovered in wild mule deer but their significance to host systems is unclear. Gastrointestinal parasites, and other parasites that shed infectious stages of their life cycle into the environment via feces, have been extensively studied in free-ranging species mostly due to convenience in sampling (Hoberg et al. 2001). However, fecal analysis may impose certain constraints in assessing the intensity of parasitic infestations. Some gastrointestinal nematodes such as *Haemonchus contortus* may stay dormant for periods of time and not shed infective stages under unfavorable conditions (Capitini et al. 1990), while other parasites have seasonal variations in shedding eggs through feces.

1.5.2 Viral agents

1.5.2.1 Bovine viral diarrhea virus (BVDV)

Bovine viral diarrhea virus belongs to the genus *Pestivirus* in the Flavivirus family and has a single stranded RNA genome. It causes a highly contagious and economically important disease in domestic cattle. Two genotypes of BVDV: BVDV 1, and BVDV 2, exist in North America. BVDV 1 strains and BVDV 2 strains are further classified into cytopathic and noncytopathic biotypes.

Bovine viral diarrhea virus infection in cattle commonly results in subclinical acute infections but can cause severe acute infection, persistent infections, enteric disease, respiratory disease, and immunosuppression (Maxie 2007). If an infected animal is gravid, there is a risk of fetal exposure resulting in early embryonic death, abortion, or congenital defects (Munoz-Zanzi et al. 2003). Infection of cows between 2-4 months of gestation results in the birth of calves which are immunologically unresponsive to that particular stain of BVDV for life. These calves remain persistently infected (PI) throughout life. Noncytopathic viruses from either genotype can cross the placenta and cause persistent infection in the fetus. The importance of the PI animals is that while they may appear clinically normal, they are viremic for life and shed large quantities of virus through sloughed-off skin cells, oral and nasal fluids, feces, urine, semen, colostrum and milk (Bolin 1995). Until recently, little was known about BVDV infection and its significance in non-bovid ungulate species in North America. Duncan et al. (2008a) demonstrated persistent infection in two neonatal white-tailed deer after experimentally inoculating the dam with noncytopathic BVDV during early pregnancy. Ridpath et al. (2008) reported that gravid white-tailed deer could develop reproductive disease including abortion, mummification and readsorption. Evidence of BVDV infection/exposure has been reported in many wild ruminants and camelids (Ridpath 2010).

1.5.2.2 Parainfluenza 3 virus (PI3)

Parainfluenza viruses are included in the *Paramyxoviridae* family and these viruses mainly cause respiratory disease such as interstitial pneumonia. These viruses contain a single stranded RNA genome. Recently, 2 genotypes were identified for cattle PI3 (PI3a and PI3b) (Horwood et al. 2008). PI3 infection has been documented in a wide range of wild ungulates around the world (Campen and Early 2001). Subclinical infection is common and virus is transmitted by aerosols. Seroprevalence has varied among locations and among deer species, ranging from 1% to 95%: 1% in sika deer (Yokoi et al. 2009), 13% in fallow deer (Giovannini et al. 1988), 20% in white tailed deer (Ingebrigtsen et al. 1986), 43% in pampas deer (Uhart et al. 2003), 58% in mule deer and 57% in wapiti (Aguirre et al. 1995), 35% in white tailed deer and 57% in mule deer (Fernando 2010), 82-84% in white-tailed deer (Sadi et al. 1991), and 95% in mule deer (Stauber et al. 1977).

1.5.2.3 Bovine herpes virus -1

Bovine herpes virus -1 (BHV-1) is an alphaherpes virus with a double-stranded DNA genome. Clinically, BHV-1 infection in cattle is represented by necrotizing rhinotracheitis, conjunctivitis, enteritis, as well as reproductive manifestations including vulvovaginitis, abortion, mastitis, or systemic infection in calves (Maxie 2007). Bovine herpes virus 1, or a similar herpesvirus, is believed to infect many wild species in North America, Europe and Africa (Aguirre et al. 1995, Kalman and Egyed 2005). However, it is not common to see clinical disease. Main sites for virus replication to occur are nasal mucosa and reproductive tract epithelium. Some studies have demonstrated serologic evidence for BHV-1 exposure in wild deer with prevalence ranging from 32% to 57% (Sadi et al. 1991, Aguirre et al. 1995, Cantu et al. 2008, Fernando 2010). Others have reported the absence of exposure or prevalence $\leq$ 15% (Ingebrigtsen et al. 1986, Frolich et al. 2006, Yokoi et al. 2009).

1.5.2.4 Mule deer lymphotropic herpes virus (mule deer-LHV)

Mule deer lymphotropic herpes virus (mule deer-LHV) is included in the genus *Rhadinovirus,* within the subfamily *Gammaherpesvirinae,* and is further categorized as belonging to the type 2 ruminant rhadinovirus (type 2 RuRV) subgroup, that are also known as the non-malignant catarrhal fever sub group. Mule deer-LHV was first reported and identified by Li et al.(2005), based on molecular characterization of gene sequences within the DNA polymerase gene. Type 2 RuRV of black-tailed deer, isolated during the same study, was identical to mule deer-LHV. Other members of the type 2 RuRV subgroup include elk-LHV, fallow deer-LHV, black-tailed deer-LHV, oryx-LHV, domestic sheep-LHV, bighorn sheep-LHV, bison-LHV, domestic goat-LHV. Forty two percent of mule deer (67/159) and 33% (8/24) of white-tailed deer were positive for mule deer-LHV in southern Saskatchewan (Fernando 2010). Immediately after initial infection, members of the *Gammaherpesvirinae* subfamily may enter latency in various sites including lymph nodes, mononuclear blood cells, ganglia, and bone marrow (Ackermann 2006). The clinical significance of type 2 RuRVs remains unknown. Squires et al. (2012) detected type 2 RuRV in conjunctiva of farmed red deer (*Cervus elaphus*) with and without ocular lesions. Little is known about transmission and prevalence of mule deer-LHV and other gammaherpesviruses in wild populations.

1.5.3 *Neospora caninum*

Neospora caninum is an intracellular protozoan parasite identified as a new species in 1988 (Dubey et al. 1988). The microscopic structure of *N. caninum* is similar to *Toxoplasma gondii*. *N. caninum* is an important pathogen of the reproductive tract of cattle, causing abortions, stillbirths and neonatal deaths. Transplacental transmission and postnatal ingestion of infective oocysts are the primary modes of spread. Domestic and wild canids such as coyotes (*Canis latrans*) are definitive hosts for *N. caninum.* Seroprevalence has been found in a variety of species including

canids, felids, equids, cervids, rodents, marine mammals and chickens (Dubey et al. 2007, Dubey and Schares 2011). Among deer species, the proportion of exposed individuals ranges from 0% to 88% (Dubey and Schares 2011). Dubey et al. (2008) documented 17% seroprevalence in mule deer and 19% seroprevalence in black-tailed deer in Washington State, USA. In contrast to *T. gondii*, isolation of viable *N. caninum* is difficult. Woods et al. (1994) found *N. caninum* in a 2 month-old black-tailed fawn (*Odocoileus hemionus columbianus*) in California as the first evidence of infection in wildlife. Later Vianna et al. (2005) isolated the infective protozoan from naturally infected white-tailed deer. Clinical disease is rare in wild animals; however, Soldati et al. (2004) reported a 3-week-old, captive fallow deer that presented with hind limb paresis related to meningoencephalomyelitis caused by *N. caninum.* Dubey et al. (1996) reported a case of non-suppurative encephalitis in a stillborn fawn of Eld's deer (*Cervus eldi siamensi*) from Paris zoo, France caused by *N. caninum*. Transmission of *N. caninum* can be maintained among wild canids and domestic animals, wild canids and wild cervids, as well as among wild cervids and domestic canids. Gondim et al. (2004) demonstrated transmission of infection to a domestic dog from naturally infected, free-ranging, white-tailed deer.

1.6 Objectives

Our primary study objectives were: 1) to estimate female reproductive performance and fawn survival rates during the first 8 months of life, and 2) to determine the incidence of exposure to selected infectious agents up to 8 months of age.

More specifically, for objective one, we evaluated whether various characteristics of the dam, such as CWD status and body condition, affected reproductive rates and fawn survival and, in addition, whether fawn traits; such as date of fawning, gender and body weight, also affected fawn survival. We also attempted to determine cause specific mortality rates for mule deer fawns and evaluated the effect of radio-marking on survival during early life. These results will provide

baseline life history parameters for models evaluating the effects of chronic wasting disease on mule deer populations.

For objective two the goal was to evaluate the exposure of mule deer, during the first eight months of life, to infectious disease agents that are transmitted primarily via direct animal-to-animal contact (agents such as bovine viral diarrhoea virus, parainfluenza 3, bovine herpes virus 1 and mule deer lymphotropic virus) and to evaluate the exposure to agents transmitted via the environment (primarily gastrointestinal parasites). The prevalence of CWD at 8 months of age was also determined but due to the long incubation period, it was predicted, prevalence would be low.

CHAPTER 2

FEMALE REPRODUCTION AND FAWN SURVIVAL OF MULE DEER IN A CHRONIC WASTING DISEASE ENDEMIC AREA IN SOUTHERN SASKATCHEWAN

2.1 Abstract

Female reproductive rates and neonatal survival rates are important life history traits that are required to model the population dynamics of a species. As part of an ongoing study of factors affecting transmission and long term population effects of chronic wasting disease (CWD), an intensive field study was initiated in a wild mule deer population in southern Saskatchewan where CWD is endemic. In 2010 and 2011, we captured female mule deer (*Odocoileus hemionus*) in winter; determined pregnancy status and body condition using ultrasound, implanted a vaginal transmitter in gravid females and then released the deer after attaching radio-collars. In the subsequent spring, we captured newly born fawns, radio-collared them and then monitored the fawns continuously until the following February or March to assess survival rates and causes of mortality. Our principal objectives were: to 1) estimate female reproductive indices and 2) evaluate fawn survival and factors affecting survival. All females examined by ultrasound scanning were pregnant and fetal rate was 1.99 ± SD 0.33 fetuses per doe. The average birth rate for all females from 2009 to 2011 was 1.29 ± SD 0.72 fawns per doe. Fawn survival rate at 8 months was 0.334 ± SD 0.047. The most common known cause of fawn mortality was predation. Having a longer body length at birth reduced the risk of fawn death for 0 - 7 day postpartum period. Members of larger litters had a higher risk of death during 8 – 30 day postpartum period. However, subclinical CWD status of dam was a poor predictor of fawn survival during the first 8 months of life. Low first year survival may result in long-term negative effects on this mule deer population.

2.2 Introduction

Fecundity and yearling survival are important life history traits which influence the population dynamics of mule deer and other ungulate species. These parameters are crucial in modeling and predicting deer population trends. In mule deer populations, the proportion of pregnant females is often > 90 %, but can differ with nutritional status of the dam, weather conditions and population density (Robinette et al. 1955, Mackie et al. 2003, Andelt et al. 2004, Bishop et al. 2009, Tatman 2009). Yearling reproduction may fluctuate significantly, depending on weather, forage availability and population density. Adult female survival and fecundity are mostly constant in older mule deer does. Older adult doe fecundity is often less sensitive to and independent of, most environmental factors (Gaillard et al. 2000).

Mule deer conceal their new born young making it hard to observe fawns just after birth in the wild. However, it is essential to study this early life stage in different populations and environments as ungulate neonatal survival may not be constant among different populations or even within a single population among years (Gaillard et al. 2000). A neonatal fawn is totally dependent on the dam prior to weaning which usually occurs at 4-6 months of age. Neonatal survival is commonly dependent on late gestational nutrition of the doe, body condition of the neonate at birth, maternal fawn rearing skills, as well as predator pressure in the environment (Gaillard et al. 2000). Fawn survival is lowest during the first 4 weeks after birth (Pojar and Bowden 2004, Johnstone-Yellin et al. 2009). Post-weaning ungulate survival is less affected by dam-related components and mortality often occurs as a result of winter starvation (Gaillard et al. 2000). Deer fawn survival studies are rare in the Canadian prairies.

Chronic wasting disease (CWD) is a prion disease of North American cervids and once contracted is invariably fatal (Williams 2005, Sigurdson 2008). Chronic wasting disease was first detected in free ranging deer in Saskatchewan, Canada more than a decade ago (Bollinger et al.

2004) and has spread across the prairies in spite of management attempts which mostly focused on herd reduction (Bollinger 2011). Effects of preclinical or clinical CWD on deer reproduction and fawn recruitment are poorly understood (Dulberger et al. 2010, Blanchong et al. 2012). After initial infection, time until clinical signs are evident can vary from weeks to years due to the characteristic slow progression of CWD, similar to that of other transmissible spongiform encephalopathies, and, thus, the consequences of maternal infection on neonatal survival are improbable (Williams 2005).

In order to estimate the long term population effects of CWD, reproductive rates and age-sex class mortality rates must be incorporated into population models. Our principal objectives were to 1) estimate female reproductive indices, and 2) evaluate fawn survival and factors affecting survival within the first 8-months of life.

2.3 Methodology

2.3.1 Study area

The study area, Antelope Creek (50.66^{o}N, 108.27^{o}W at center) covers approximately 248 km^{2} and is located immediately south of the South Saskatchewan River in southwestern Saskatchewan, Canada (Figure 2-1). It is primarily composed of a series of complex coulee systems which radiate away from the river valley.

Antelope creek is in the mixed grassland ecoregion of the prairie ecozone. It has a semi-arid climate with an annual mean precipitation of 352mm and annual mean temperature of 4.0^{o}C. Summers are warm and short while winters are severe, typically lasting more than 6 months. Glacial lake plain comprised of mostly native grassland and agricultural cropland is dominant in the Antelope Creek area along with a moderate number of steep slopes and shrubby areas in depressions (Scott 1995, Acton et al. 1998). The native grassland is a mixture of short grass varieties including blue grama grass (*Bouteloua gracilis*), and taller types such as wheat grass

(*Agropyron* spp.), speargrass (*Stipa comata*) and June grass (*Koeleria macrantha*). Wolf willow (*Elaeagnus commutata*), wild prairie rose (*Rosa arkansana*), Saskatoon berry (*Amelanchier alnifolia*), chokecherry (*Prunus virginiana*), prickly pear cactus (*Opuntia polyacantha*) and creeping juniper (*Juniperus horizontalis*) are commonly found. In moist sheltered draws, a limited distribution of tree species including balsam poplar (*Populus balsamifera*), black cotton wood (*Populus trichocarpa*), willow (*Sali*x spp.) and trembling aspen (*Populus tremuloides*) are present, together with grass and shrub communities. The predominant crop types cultivated include winter wheat, spring wheat, durum, oat, barley, canola, mustard, lentils and peas (Government of Saskatchewan 2010).

Wild ungulates consist primarily of mule deer with lesser numbers of while-tailed deer, pronghorn antelope and infrequent moose. Potential predator species are coyote (*Canis latrans*) and red fox (*Vulpes vulpes*) (Acton et al. 1998). Bobcats (*Lynx rufus*), cougars (*Puma concolor*) and golden eagles (*Aquila chrysaetos*) are found in the area rarely (G. Wobeser personal communication).

Chronic wasting disease prevalence in wild mule deer in the Antelope Creek study area was greater than 25% during this study (Bollinger 2011).

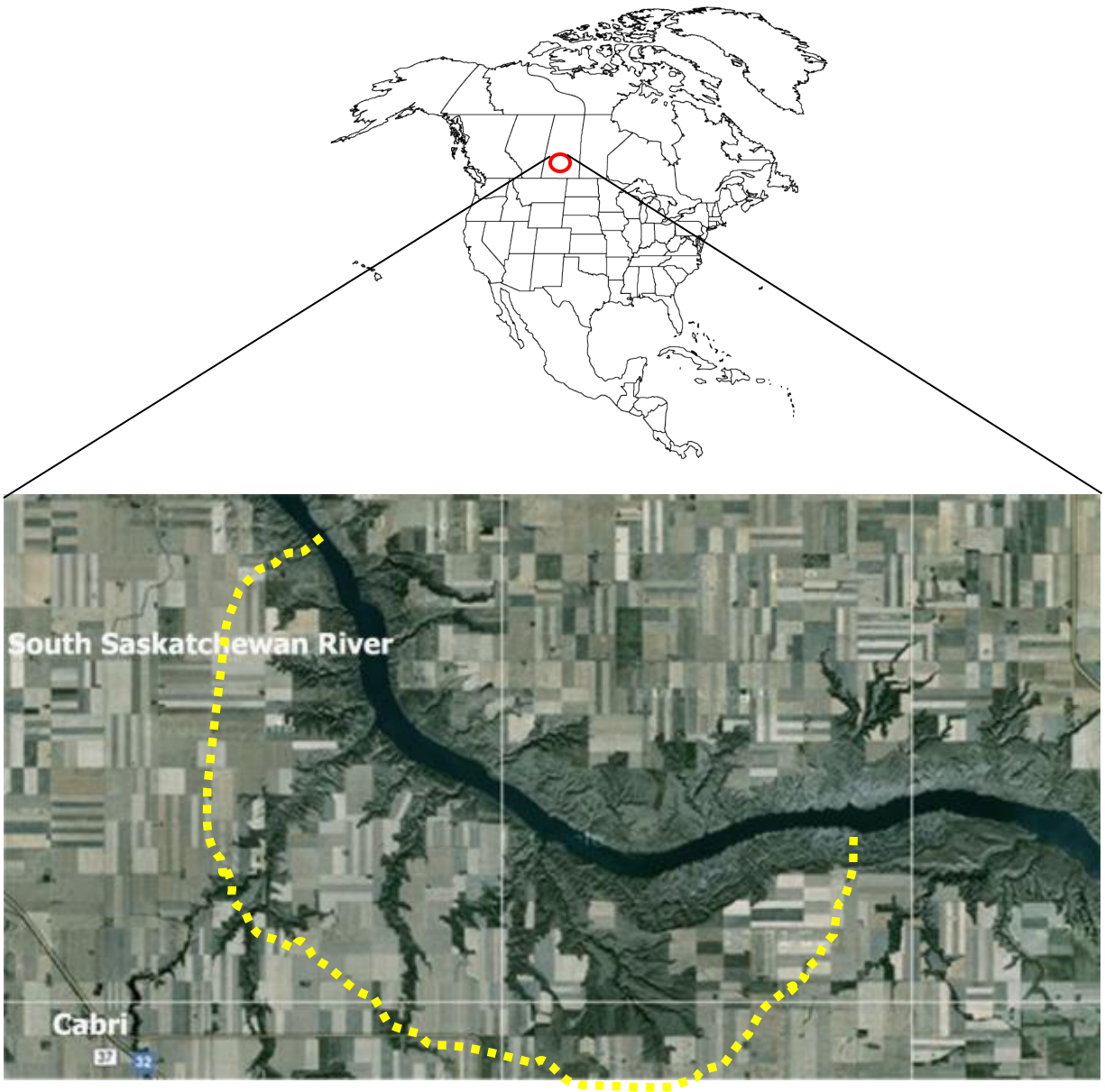

Figure 2-1: Antelope Creek study area (dotted line) in southwestern Saskatchewan, Canada

2.3.2 Females

2.3.2.1 Capture

In February-March of 2009 to 2011, inclusive, adult does were captured using a net gun fired from a helicopter (Barrett et al. 1982). After capture, deer were hobbled, blind-folded and ferried by air to a central processing location where they were anaesthetized with a combination of tiletamine hydrochloride and zolazepam hydrochloride (3 - 4 mg/kg) (Telazol®, Fort Dodge Animal Health, IA, USA) and xylazine hydrochloride (1.5 - 2 mg/kg) (Rompun®, Bayer Inc., ON, Canada) as an intramuscular injection. A tonsillar biopsy was obtained from each deer in order to perform immunohistochemistry for infectious CWD prions (Wolfe et al. 2002). After reversing with atipamezole hydrochloride (0.2 mg/kg) (Antisedan®, Pfizer Animal Health, NY, USA), females were ferried back to their approximate capture location and released.

2.3.2.2 Pregnancy and body condition measurement

Trans-abdominal ultrasound was performed in 2010 and 2011, using a portable ultrasound unit (Micromaxx, SonoSite Inc. Bothell, WA, USA) with a 5 - 2MHz curved-array transducer to detect pregnancy status and number of fetuses in each adult (> 1 year of age) doe. We used fetal heart beat observed from ultrasonography to determine if each fetus was alive at the time.

To measure nutritional status of adult does, a body condition scoring system based on palpation of withers, ribs and rump was used (Gerhart et al. 1996b, Cook et al. 2007). Adult females were assigned a body condition score (BCS) ranging from 1-6; score 1 was assigned to those deer in poorest condition and score 6 was used for obese animals (Cook et al. 2007). Additionally, subcutaneous rump fat thickness was measured to the nearest 0.1 cm using ultrasound (T180, SonoSite Inc. Bothell, WA, USA, L38/ 10-5 MHz linear array transducer) with electronic calipers (Stephenson et al. 1998, Cook et al. 2007). Maximum fat thickness (MAXFAT) was determined for each deer following Stephenson et al. (1998). Hair was plucked

and ultrasound imaging gel was used to improve transducer contact with skin prior to scanning. Body mass (BM) of each doe was also determined to the nearest 0.1 kg using a spring weigh scale suspended from a tri-pod. Chest girth circumference was measured to the nearest 0.5cm for each deer.

For further analysis of female nutritional status, BM and BCS were combined as a product to create a body reserve index (BRI) (Cook et al. 2007). Chest girth was used to calculate BM using the formula BM= 1.2925 × (girth) - 60.80 and scaled MAXFAT was estimated using formula: scaled MAXFAT=MAXFAT/[0.142 × (BM $^{0.630}$)] (Cook et al. 2010). We assumed that the BCS for the total body was also similar to condition at rump (rBCS). ScaledMAXFAT and rBCS were used to calculate a scaledLIVINDEX. When MAXFAT ≥ 0.3 cm, we obtained scaledLIVINDEX by the formula: scaledLIVINDEX = 11.35 × (scaledMAXFAT) + 5.63, and when MAXFAT < 0.2 cm, the formula: scaledLIVINDEX = 3.869 × rBCS – 2.71 was used. If 0.2 ≤ MAXFAT < 0.3, scaledLIVINDEX = ([3.869 × (rBCS) – 2.71] + [11.35 × (scaledMAXFAT) +5.63])/2 was used. Ingesta free body fat percentage (IFBF %) is the total body fat as a percentage of total body mass excluding gut contents and is a measure of body condition in ungulates (Stephenson et al. 1998, Cook et al. 2007). IFBF% was calculated using scaledLIVINDEX by the formula: IFBF%= - 0.16 + 1.010 × (scaledLIVINDEX) (Cook et al. 2010).

2.3.2.3 Vaginal Transmitter Implantation and Radio collaring

In 2010 and 2011, pregnant females were implanted with a thermo-sensitive vaginal implant transmitter (VIT) (M3930, Advanced Telemetry Systems, Isanti, MN, USA) using a previously documented protocol (Bishop et al. 2007). Each vaginal implant was pre-programmed to switch to 80 beats per minute (bpm) from 40 bpm when the implant was expelled, typically during a birthing event. The Precise Event Transmitter (PET) mode of the VIT used a sequence

of 8 beeps to indicate time elapsed, to the nearest thirty minutes, since the expulsion. In 2009, the first year of the study, we did not perform trans-abdominal ultrasonography for pregnancy detection or use VITs.

Adult females were marked with either a GPS (Global Positioning System) or VHF (Very High Frequency) radio-collar (Models 3300L, 4400M, 7000SU, LMRT-4, Lotek Wireless Inc. ON, Canada). Age of each doe was determined using tooth eruption and wear (Robinette et al. 1957). Female deer were classified as yearling (between 1-2 years) and adult (> 2 years) for analysis.

2.3.3 Fawns

2.3.3.1 Capture

Following winter capture, radio-collared does were monitored from the ground twice each week until late May, using a hand held Yagi antenna and telemetry receiver. Beginning in the last week of May, each VIT signal was checked every 1-2 days for expulsion signals. When a post-expulsion signal was detected, the signal sequence was decoded to obtain the time gap between expulsion and detection (Age at capture). To allow adequate dam-fawn imprinting to occur before any intervention, fawns were not approached until at least 5 hours after birth (Haskell et al. 2007). The fawning date for each doe in 2010 and 2011 was determined using a combination of doe behaviour and VIT transmission data. Each doe and VIT (in 2010 and 2011) was tracked to locate where fawning occurred. Doe behaviour was also observed for probable location of neonatal fawns in area (Carstensen et al. 2003). A thorough search for new born fawns was carried out initially in the vicinity of the dam, and, when that failed, around the expelled VIT location. These areas were searched by 2 to 3 persons for a maximum of 2 hours on the first day, followed by 1.5 hours per day for up to 3 consecutive days. In 2009, when VITs were not used, parturient radio-collared does were tracked and located every other day and the

area they were found was searched extensively for neonates. Newborn fawns that were encountered during ground searches and born to uncollared does also were radio-collared in order to increase the number of marked fawns under observation. Fawns were captured by approaching them quietly from behind and then grasping them with latex gloved hands. They were then blind-folded and secured in a pillow case. They were sexed, weighed (to nearest 0.1kg), and measured for total body length from tip of the nose to last vertebra of tail, chest girth behind elbow joints of fore limbs as a body circumference (to nearest 0.1cm), new hoof growth of right front and right hind limbs (to nearest 0.5mm at the base of the hoof). Blood (2-3 ml) was collected either from jugular or cephalic vein for a parallel study. During processing every crew member wore disposable latex gloves and processing time was kept as short as possible.

2.3.3.2 Radio Telemetry

Each fawn was fitted with an expandable VHF radio collar (M4210, Advanced Telemetry Systems, Isanti, MN, USA) that switched to a mortality signal (80 beats per minute) from a live signal (40 beats per minute) if the collar was motionless for 4 hours. After applying a numbered metal ear tag (Ketchum Manufacturing Inc., ON, Canada), neonates were released at the original capture location. From June to end of September, radio signals from fawns were monitored every day, and then twice a week until next winter capture in February/March of following year.

2.3.3.3 Mortality Detection

Whenever a mortality signal was detected, the radio collar was located as soon as possible in order to assign a proximate cause of mortality using carcass remains and evidence at the site. Presence of evidence such as hair, bone shards, and/or blood stains on vegetation, or blood on the collar, was recorded during site examinations. If a partial or whole carcass was recovered, it was frozen and a complete necropsy examination was performed later at Western College of Veterinary Medicine, University of Saskatchewan, SK, Canada.

The cause of death was classified into major categories: predation, accident, starvation, disease and unknown. Predation was judged to have occurred when: blood was present on the collar, vegetation and ground in the absence of a carcass; or, when internal hemorrhage was present together with bite marks when the entire or a portion of a carcass was found (White 1973). Death was classified as accidental if multiple bone fractures were present along with skin bruising and extensive hemorrhage, or when a carcass was found in the river, in creeks or in deep crevices where the fawn could not escape, or when hunters reported shooting the fawn. When the abomasum was empty of colostrum or milk, the subcutis was dry and there was absence of lesions belonging to any other category, the cause of death was classified as starvation. At this early life stage, we assumed starvation was the result of abandonment. If post-mortem examination and laboratory testing (histopathology, bacterial culture, polymerase chain reaction for viruses) revealed findings indicating underlying infectious disease, death was classed as infection- related. When we could only find bone shards, tufts of hair, bite marks on collar, or bone, tissue or heavily scavenged remains without blood, the cause of death could not be determined and the cause of mortality was classified as unknown. In instances when the radio collar signal was lost suddenly for unknown reasons or only a clean radio-collar was found in mortality mode with no accompanying proof of death, we assumed it to be a dropped collar and the animal was excluded from further analysis. The capture and animal handling protocol was approved by the Animal Care Committee of University of Saskatchewan (Permit number: 20050135).

2.3.4 Weather Data Collection

In order to assess the effect of winter on doe body condition, winter severity measures were examined in 2010 and 2011. All weather data used in analysis were obtained from Environment Canada online data archives (Environment Canada 2011). The nearest weather station with the

most complete historical and recent weather information was at Swift Current, Saskatchewan, located approximately 60 km southeast of the study area. Days with minimum temperature less than -20°C and days with a snow depth equal to or higher than 10 cm were used as criteria which indicated severe winter conditions. The cut-off temperature of -20 °C used here was just below the effective critical temperature for mule deer which is -18 °C (Mautz 1985). Effective critical temperature is the threshold temperature at which an animal must increase their body metabolic rate to maintain body temperature (Farnes 2002). Following DeLgiudice et al.(2002) and Skelton (2010), a winter severity index (WSI) was calculated using the sum of the days the minimum daily temperature fell below -20 °C and the days snow depth was $\geq$ 10 cm. For analysis, winter was considered to be between November 1st and March 31st. Summer precipitation was documented to be strongly predictive of forage availability in winter range (Farnes 2002). Total precipitation from May 1st to October 31st thus was used as an indicator of forage availability in the following winter. Thirty-year weather values for total days with minimum temperature, total days with snow depth and summer precipitation, available from 1970 to 2000 at Swift Current weather station (Environment Canada 2011), were used to compare 2010 and 2011 winter severity indices with long term averages.

2.3.5 Statistical Analysis

SAS 9.2 software (SAS Institute Inc., NC, USA) was used for survival analyses and all other analyses were performed using SPSS (IBM SPSS statistics 19, IBM Corporation, NY, USA).

2.3.5.1 Doe body condition

Initially, all variables describing body condition, continuous variables (weight, chest girth, body length, rBCS, BRI) and categorical variables (scaledLIVINDEX by median, age group, CWD status, capture year, IFBF%), were explored with descriptive statistics, and tested for

normality and outliers. Then, to minimise the number of variables entering multivariable models and to ensure parsimony, correlations were assessed on dependant variables IFBF % and rBCS. Normally and non-normally distributed independent variables were assessed for probable correlations using Pearson or Spearman correlation coefficients, respectively. According to significance level at 0.05, minimally correlated covariates were selected to assess effects on IFBF % and BCS as measures of doe body condition using Mann-Whitney U test on pairwise comparisons.

2.3.5.2 Birth Attributes

We assumed birth of twins or triplets to be a single birthing event. Age at capture in days was determined by decoding the PET code of expelled VITs in 2010 and 2011, assuming VIT expulsion and parturition occurred simultaneously. For fawns born in 2009, or fawns born to does with VIT malfunction and for fawns from uncollared does, new hoof growth after birth was used for calculation of age at capture assuming 0.4 mm of new hoof growth per day following Robinette et al.(1973). Birth mass was estimated using capture mass, calculated age at capture and assuming a daily weight gain of 0.29 kg during first 12 days after birth (Robinette et al. 1973). We estimated birth date using capture date and age at capture. As our sample size was very small (n=5) for triplets, we grouped fawns in to 2 groups, according to litter size, as singletons or litter size ≥ 2 (twins and triplets) in analysis.

After assessing relevant assumptions and descriptive statistics, we performed one way ANOVA to compare birth attributes (estimated birth weight, capture weight, chest girth, total body length and rectal temperature), between 3 capture years, 2 sex groups, 2 litter size groups, and dams affected or not with CWD. One way ANOVA was also used to compare mean estimated birth date among 2010 and 2011. We performed Scheffe's test as a post-hoc test to compare means between any significant pair-wise year combinations resulting from ANOVA.

2.3.5.3 Fawn Survival analysis and modelling

Individual survival time was calculated from estimated date of birth to last date a fawn radio-collar was heard emitting an "alive" signal. Fawns for which radio-collar signals were lost during the study or for which only a clean collar was found in mortality mode, were censored from all survival analysis procedures. We also categorised fawns in to two groups, pre-peak or post-peak, depending on whether fawn was born before or after the overall mean birth date (June 12). We analysed univariate effects of sex, litter size, capture year, age at capture, Julian date of birth, variables related to fawn condition at birth (estimated birth mass, chest girth at birth, total length at birth), dam characteristics: dam CWD infection status, covariates of dam body condition (IFBF%, BCS, scaledLIVINDEX and BRI categorised by median) and doe age group on fawn survival. Proportionality assumption was assessed by including time-dependant effect covariates for each variable in univariate as well as multivariate models (Collett 2003, Allison 2010). In univariate analysis for categorical variables, PROC LIFETEST method (SAS version 9.2) on Kaplan Meier estimate (Kaplan and Meier 1958) was used, taking the non-parametric log-rank (for proportional variables) or Wilcoxon (for non-proportional variables) as tests of equality across strata (Collett 2003). The semi-parametric Cox proportional hazard model (Cox 1972) was applied for continuous variables using PROC PHREG for univariate models as well as for final multivariate model building. The univariate covariates which had a significance of $P < 0.25$ were selected as candidates for final multivariate model testing. For any significant main effects in the final model, possible interaction terms were assessed for significance at $\alpha=0.05$ level. However; if any of the covariates were non-proportional, the final model was stratified over levels of non-proportionate variables. Ties were handled using the exact method in PROC PHREG and the backward model selection method in SAS was utilised. Akaike's Information Criterion with second order bias correction for small samples (AICc) was used to compare

between candidate models, and Akaike weight (w_i) was used to determine relative importance of each model (Burnham and Anderson 2002). The above analyses were performed separately for overall survival for pooled data from birth, up to 6 months and for four age intervals: day 0-7 (week 0-1), day 8-30 (week 1- month 1), day 31-90 (month 1-3) and day 91-180 (month 3-6) from 2009 to 2011. We did not model survival beyond 6 months as no fawn deaths occurred in any capture year after 6 months of age and, thus, survival remained constant thereafter.

2.3.5.4 Handling and radio-collar effects on neonatal survival

We expressed radio collar mass (68 g each for all fawns collared within 3 capture years) as a percentage of body mass at capture by simply dividing collar mass (kg) by capture mass (kg). According to the Canadian Council on Animal Care- guideline 28, on the care and use of wildlife, this ratio should not exceed 5% in free ranging and captive wild vertebrates (Canadian Council on Animal Care 2003). In this study, we assessed whether fawn survival was affected by a radio collar: body mass at capture ratio $\geq$ 2%, as for all the fawns the ratio was well within the guidelines. We used PROC LIFETEST in SAS to see effects of radio collar weight: body mass ratio.

Further to evaluate any possibility of effects of capture, handling and radio collars on neonate mortality, we analysed effects of capture related covariates: handling time, estimated age at capture, as well as birth attributes (estimated birth weight, chest girth and total body length of neonates) on neonates that died within 4 days of capture and that survived longer than 4 days post-capture. Four days post-capture was selected as the most critical time for possible natural or radio-collaring and processing related abandonment as described by (Powell et al. 2005). The Mann-Whitney U test was used to compare between two neonatal groups: animals that died within 4 days post-capture or those that survived this period.

2.3.5.5 Fawn recruitment

We defined annual recruitment in to the population as the average number of fawns surviving to 6 months of age per adult doe. As the average date of birth for the pooled data was June 12, we used the total number of fawns on Dec 12 of each year when they were approximately 6 months and total number of does alive on Dec 12 to calculate recruitment rates. Further, we calculated recruitment separately for CWD infected and uninfected females for each capture year. We did not know if the uncollared dams of radio-collared fawns were alive in winter and thus such dams and their radio-collared fawns which had survived to 6 months, were not included in these calculations.

2.4 Results

2.4.1 Doe body condition

In 2010 and 2011, we measured body condition of 40 and 44 female deer, respectively. Body weight was positively correlated with chest girth (r = 0.255, P = 0.019, n = 84). Ingesta free body fat percentage was correlated with scaledLIVINDEX (r_s = 0.687, P= 0.000, n = 31), year (r_s = -0.812, P = 0.000, n = 32), and but not doe subclinical CWD status (r_s = -0.366, P = 0.05, n = 30). Because it is interesting biologically to know if subclinical CWD infection status and capture year affect body condition, these variables were considered further. Female age group, capture year and doe subclinical CWD status were assessed as predictor variables on outcome variables IFBF % and BCS using Mann-Whitney U test.

Median IFBF % did not differ between adult and yearling age groups (Z = -0.952, P = 0.341) or CWD status (Z = -1.973, P = 0.05). Similarly, BCS did not differ across age groups (Z = -0.846, P = 0.398) or doe subclinical CWD status (Z = -1.149, P = 0.251). However, IFBF % (Z = -4.519, P = 0.000) and BCS (Z = -2.628, P = 0.009) differed between the winters of 2010 and

2011. Capture year 2011 had lower mean rank than 2010 for both IFBF % and BCS, indicating a lower female body condition in 2011.

2.4.2 Female Reproduction

We captured 84 mule deer females (n = 40 in 2010 and n = 44 in 2011) and checked for pregnancy using transabdominal ultrasonography. In 2010, 88% (n =35) were adults. In 2011, 27% (n = 12) were yearlings and 73% (n = 32) were adults. There were two capture related female deaths each year, within 2 weeks post-capture.

In both years, all adult and yearling females were pregnant (100%). The proportion of does that carried singleton, twin and triplet fetuses in 2010 and 2011 are shown in figure 2-2. Overall, the mean number of fetuses per doe was 1.99 ± SD 0.33 (167 fetuses, 84 does) (Table 2-1; Figure 2-2), with no apparent difference between capture years. In both years, older females had consistently greater fetal rate compared to yearling females.

Table 2-1: Fetal rate (average number of live fetuses per female in February/March) in yearling and adult female mule deer captured in 2010 and 2011 as determined by transabdominal ultrasonography.

	2010			2011			Both years		
	Yearling	Adult	Overall	Yearling	Adult	Overall	Yearling	Adult	Overall
Total fetuses	11	67	78	23	66	89	34	133	167
Total females	6	34	40	12	32	44	18	66	84
Fetal rate	1.83	1.97	1.95	1.92	2.06	2.02	1.89	2.02	1.99

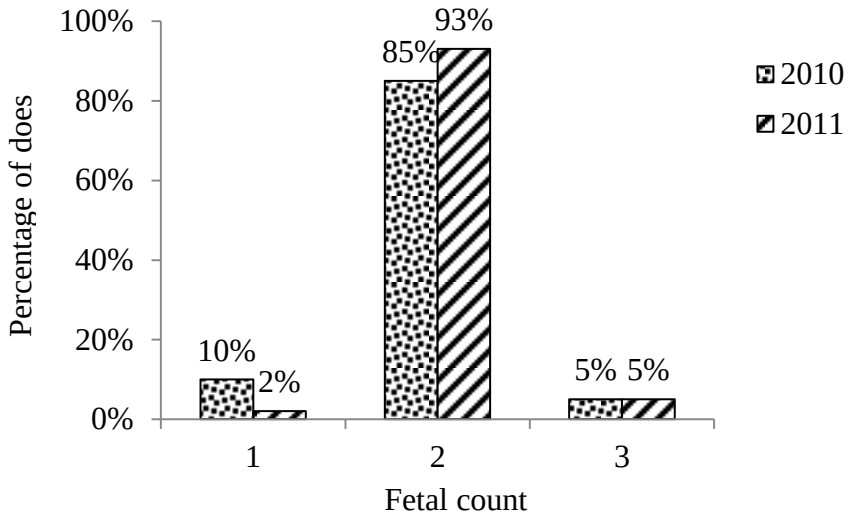

Figure 2-2: Proportion of mule deer females with one, two or three fetuses as determined by transabdominal ultrasonography in 2010 and 2011.

2.4.3 Birth attributes

From 2009 to 2011, we captured 118 neonates (38 in 2009, 41 in 2010, and 39 in 2011). In 2010, we found 2 dead neonates in the vicinity of collared does and in 2011 we found one dead fawn with a live sibling. Of the fawns captured as neonates, 78% (n = 92) were from collared females: 58% (22/38), 80% (33/41), and 95% (37/39) in 2009, 2010 and 2011, respectively. The remaining fawns were born to unknown, non-collared females, and were encountered during ground searches. In 2010 and 2011, years in which vaginal implant transmitters were used, 76% (61/80) of the fawns were born to females implanted with VITs. In addition to collared fawns, as part of a parallel behavioural study we observed presence of live fawns that were neither found nor collared within the fawning period but were believed to have been born to collared females. We confirmed the maternal relation of a particular collared dam to such fawns either by observing suckling or by seeing an unmarked fawn with the relevant doe on two or more occasions during observations. In 2010, 7 uncollared fawns were observed with 5 collared does and 11 uncollared fawns were seen with 10 collared does in 2011. These uncollared fawns were included in birth rate calculations for each year.

The mean birth rate for all the females from 2009 to 2011 was 1.29 ± SD 0.72 fawns per doe (139 fawns, 108 females), including non-collared fawns observed with collared females as well as collared fawns from non-collared females (Table 2-2). In 2009, 2010 and 2011, birth rates were 1.48 (40 fawns, 27 females), 1.23 (48 fawns, 39 females), and 1.21 (51 fawns, 42 females) fawns per doe respectively (Table 2-2). The birth rate in 2009 was comparatively higher than the following two years, overall and for CWD infected and non-infected females considered separately. There was an apparently lower birth rate of 1.13 fawns per doe in CWD infected females in 2011 than in 2010 (1.6 fawns per doe), which probably was partially contributed by the 3 fold larger number of CWD infected females in 2011(n=15). This was likely associated

with increasing CWD prevalence in the study area. Among CWD non-infected females, birth rate was apparently similar in 2010 and 2011.

During the 3 capture years, we captured 33 sets of twins (11 sets each in 2009, 2010, and 2011) and 47 singletons (16 singletons in each of 2009 and 2010, 15 singletons in 2011). In 2010, we managed to capture and collar a set of triplets, but in 2011 we could only capture 2 fawns of likely triplet siblings. The uncollared member of 2011 triplet set was observed on multiple occasions moving with the dam after the fawning season.

From 2009 to 2011, fawning date ranged from May 27th to July 6th and peaked during the 2nd and 3rd weeks of June (Figure 2-3). The mean estimated birth date was June 12th when data were pooled for all 3 years. In 2010 and 2011, years when we used VITs, mean birth dates were June 11 and June 13, respectively, and but these dates were not significantly different (P = 0.338).

The mean estimated birth weight for all years was 3.1 ± SD 0.86 kg, with a minimum of 1.2 kg and maximum of 4.9 kg (Table 2-3). The mean birth weight of singletons, twins and triplets was 3.2 ± SD 0.88 kg, 3.0 ± SD 0.86 kg and 2.8 ± SD 0.54 kg, respectively. There was no difference in estimated birth weight between singletons and litter mates of twins or triplets ($F_{1, 113}$ = 2.09, P = 0.151). Mean estimated birth weight differed between years ($F_{2, 112}$ = 5.6, P = 0.005) but not between sexes ($F_{1, 113}$ = 0.791, P = 0.376) (Table 2-4). Mean estimated birth weight in 2010 was greater than in 2009 (P = 0.037, 95% CI= 0.023-0.957) and 2011(P = 0.010, 95% CI= 0.113-1.034). During capture years 2009 and 2011, fawns had similar mean estimated birth weights (P=0.910, 95% CI= -0.559 - 0.392).

Table 2-2: Annual birth rates (number of live fawns per doe) from 2009 to 2011 for chronic wasting disease (CWD) infected and uninfected females. CWD infection status of the dam was diagnosed by immunohistochemistry on tonsillar biopsy. Inconclusive CWD status includes deer with inadequate lymphoid tissue obtained during tonsillar biopsy. *n* = sample size of females. (*) = birth rate not calculated for CWD infected females in 2009 due to very low sample size.

Capture year	2009	2010	2011	Overall
CWD infected females:				
• Total fawns	2	8	17	27
• *n* (with fawns)	1	5	11	17
• *n* (without fawns)	0	0	4	4
• Birth rate	*	1.6	1.13	1.29
CWD uninfected females:				
• Total fawns	19	31	25	75
• *n* (with fawns)	12	20	17	50
• *n* (without fawns)	0	8	3	11
• Birth rate	1.58	1.11	1.25	1.23
CWD inconclusive females:				
• Total fawns	2	1	7	10
• *n* (with fawns)	1	1	5	7
• *n* (without fawns)	0	0	0	0
Uncollared females:				
• Total fawns	17	8	2	27
• *n*(with fawns)	13	5	2	20
Annual:				
• Total fawns	40	48	51	139
• Total dams	27	39	42	108
• Birth rate	1.48	1.23	1.21	1.29

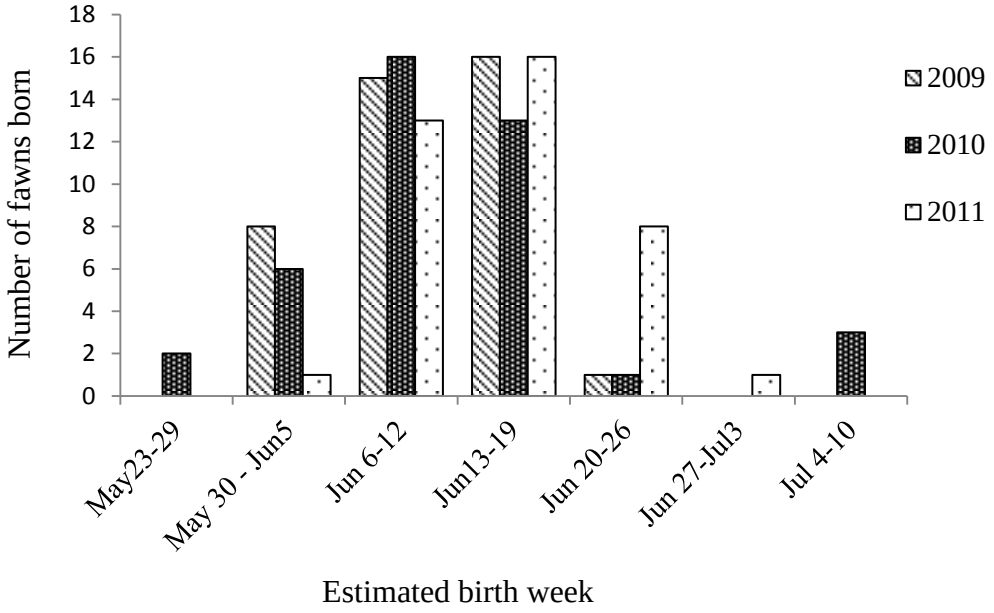

Figure 2-3: Total number of mule deer born each week during fawning seasons from 2009 to 2011. Birth dates were estimated relative to capture date using estimated age at capture by the number of days the VIT was expelled before capture or new hoof growth after birth.

Table 2-3: Descriptive statistics on birth attributes for neonatal mule deer captured from 2009 to 2011. *n* = total number of fawns used in analysis, SD = standard deviation.

Birth attribute	*n*	Minimum	Maximum	Mean (SD)
Estimated birth weight (kg)	115	1.2	4.9	3.1 (0.9)
Capture weight (kg)	118	1.7	9.4	3.7 (1.1)
Chest girth (cm)	116	28.0	42.0	35.3 (3.1)
Total body length (cm)	117	27.6	81.0	65.8 (6.8)
Average new hoof (mm)	116	0.0	7.5	3.1 (1.1)
Rectal temperature (°C)	115	33.9	41.4	38.7 (1.2)

Capture weight varied among years ($F_{2, 115}$= 3.189, P = 0.045) and litter size ($F_{1, 116}$= 10.978, P = 0.001) but not between sexes ($F_{1, 116}$= 0.375, P = 0.541) (Table 2-4). Mean chest girth ($F_{1, 114}$ = 0.013, P = 0. 910) and total length ($F_{1, 115}$ = 0.776, P = 0.380) did not vary between sexes (Table 2-4). Mean chest girth ($F_{1, 114}$ = 6.814, P = 0.010) and total body length ($F_{1, 115}$ = 9.195, P = 0.003) differed among singletons and fawns born as twins or triplets, with higher values in singletons. Mean chest girth ($F_{2, 113}$= 5.210, P = 0.007) differed among years but not mean total length ($F_{1, 114}$ = 1.617, P = 0.203). Comparisons among years, sexes and litter sizes for rectal temperature are shown in table 2-4.

Table 2-4: Summary of one way ANOVA for birth attributes of mule deer neonates between sex and among capture years 2009, 2010, and 2011. Significance level (α) = 0.05.

Birth attribute	Among 3 capture years	Between sexes	Between singletons and twin/triplets
Estimated birth weight (kg)	$F_{2,112} = 5.60$ $P = 0.005$	$F_{1,113} = 0.79$ $P = 0.376$	$F_{1,113} = 2.09$ $P = 0.151$
Capture weight (kg)	$F_{2,115} = 3.18$ $P = 0.045$	$F_{1,116} = 0.37$ $P = 0.541$	$F_{1,116} = 10.97$ $P = 0.001$
Chest girth (cm)	$F_{2,113} = 5.21$ $P = 0.007$	$F_{1,114} = 0.01$ $P = 0.910$	$F_{1,114} = 6.81$ $P = 0.010$
Total length (cm)	$F_{2,114} = 1.61$ $P = 0.203$	$F_{1,115} = 0.77$ $P = 0.380$	$F_{1,115} = 9.19$ $P = 0.003$
Rectal temp (°C)	$F_{2,112} = 8.92$ $P = 0.000$	$F_{1,113} = 0.01$ $P = 0.945$	$F_{1,113} = 2.65$ $P = 0.106$

Female subclinical CWD infection status did not affect any of the birth attributes: estimated birth weight ($F_{1, 80}$ =1.29, P = 0.259), capture weight ($F_{1, 82}$ = 0.091, P = 0.763), chest girth ($F_{1,81}$ =0.024, P = 0.878), total length ($F_{1,82}$ =1.289, P = 0.259), or rectal temperature ($F_{1, 80}$ =2.71, P = 0.103).

2.4.4 Fawn survival

Sixty percent (71/118) of radio-collared fawns died within the first 8 months of life, 26% (30/118) were known to be alive until 8 months of age, and the fate of 14% (17/118) of fawns could not be confirmed (Table 2-5). We censored 5, 4, and 8 fawns from all survival analysis in 2009, 2010 and 2011, respectively, because of loss of radio-signal or because we could not be certain that the radio-collar had not been rubbed off of an otherwise healthy animal as there was no evidence of death and only a clean collar was found. Within 7 days of birth, 14 fawns died. Thirty five fawns were alive for more than 7 days, but did not survive beyond 30 days. Sixteen and 7 radio-collared fawns succumbed between 31-90 days and 91-180 days postpartum, respectively. No fawn deaths occurred after 180 days up to winter capture in the following year (February/March) in any of the capture years.

Overall 8 month survival rate was 0.334 ± SE 0.047 with a median survival time of 42 days. In 2009, 2010 and 2011, survival rates were 0.347 ± SE 0.083, 0.335 ± SE 0.078 and 0.340 ± SE 0.083 respectively (Table 2-6). The period during which survival was lowest (mortality was highest) was between 8 and 30 days after birth in 2009 and 2011 as well as when data were pooled from all 3 years. Survival was lowest during the 31-90 day postpartum period in 2010 (Table 2-6, Figure 2-4).

Table 2-5: Summary of mule deer fawns radio-collared in Antelope Creek from 2009 to 2011. *n* = sample size of fawns.

Capture year	2009		2010		2011		Overall	
	n	%	*n*	%	*n*	%	*n*	%
Total died	22	58	25	61	24	62	71	60
Total survived	11	29	12	29	7	18	30	26
Censored	5	13	4	10	8	20	17	14
Total collared	38	100	41	100	39	100	118	100

Table 2-6: Mean survival probability (standard error) of mule deer fawns for various time periods following birth from 2009 to 2011.

Period	2009	2010	2011	All years
0-7 day	0.947(0.037)	0.901(0.047)	0.744(0.069)	0.862(0.032)
8-30 day	0.600(0.086)	0.739(0.0745)	0.546(0.093)	0.634(0.049)
31-90 day	0.722(0.106)	0.672(0.095)	0.812(0.124)	0.726(0.061)
91-180 day	0.846(0.100)	0.750(0.108)	1.00(0.00)	0.833(0.062)
181-240 day	1.00(0.00)	1.00(0.00)	1.00(0.00)	1.00(0.00)
Overall (0 – 180 day)	0.347(0.083)	0.335(0.078)	0.340(0.083)	0.334(0.047)

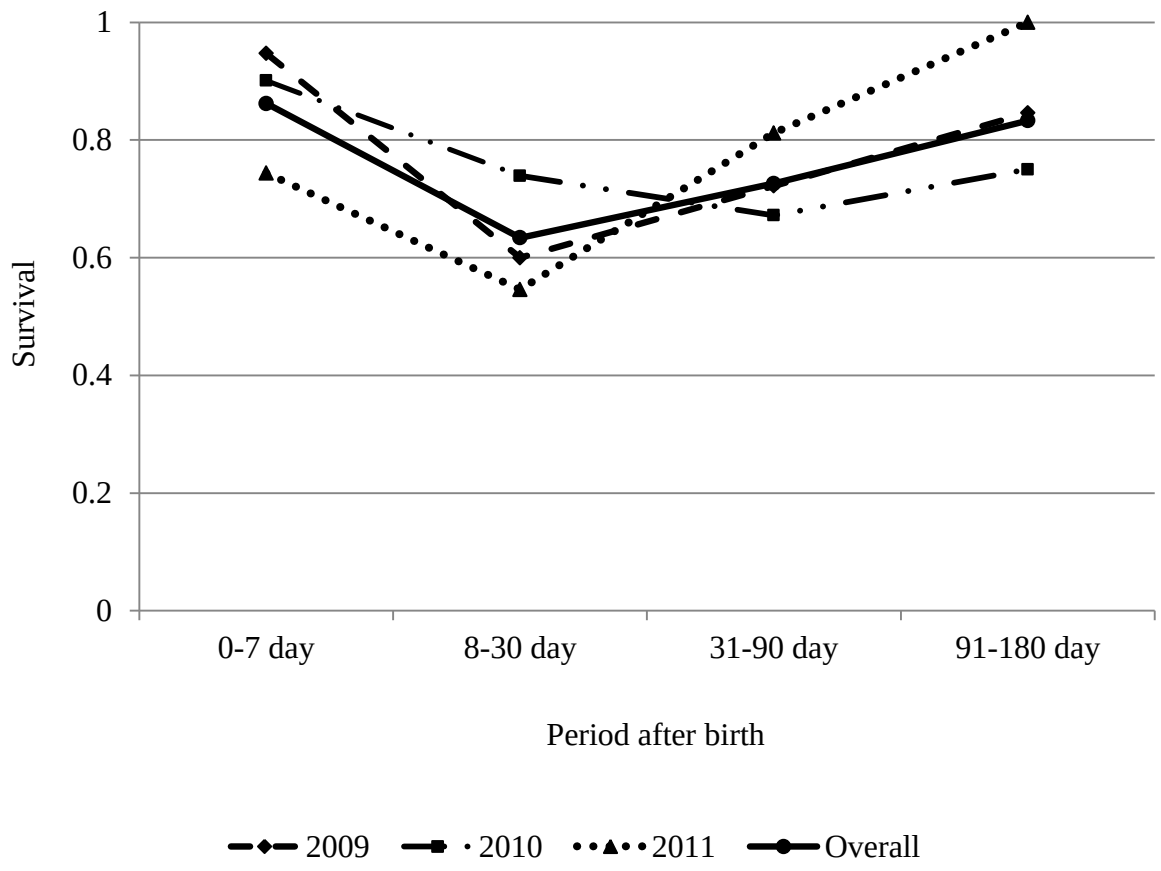

Figure 2-4: Survival probability for mule deer fawns for various time periods (0-7 day, 8-30 day, 31-90 day, and 91-180 day) following birth from 2009 to 2011.

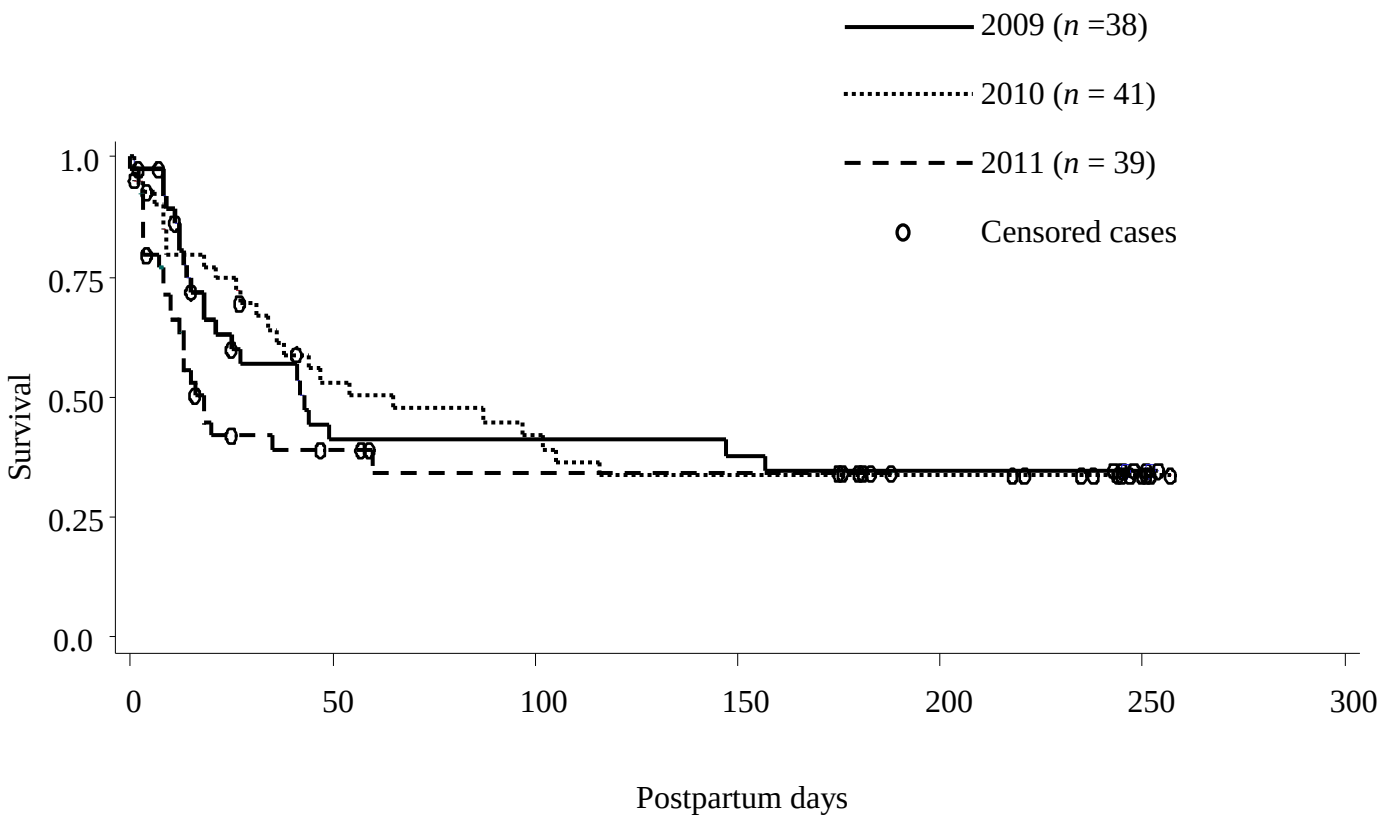

Figure 2-5: Survival probability for mule deer neonates radio-collared in spring 2009, 2010 and 2011.

2.4.4.1 Univariate analysis for fawn birth attributes on survival from birth to 8 months

On univariate analysis, annual survival did not differ among capture years based on log rank test ($\chi_2^2 = 1.71$, $P = 0.425$) (Figure 2.5). Median survival for male fawns was 43 days and that of female fawns was 41 days. Annual survival for females (0.306 ± SE 0.066) and males (0.361± SE 0.067) did not differ ($\chi_1^2 = 0.066$, $P = 0.796$). Within the three capture years, 41 singletons, 72 twins and 5 triplets were radio-collared. Median survival time for singleton, twin and triplet fawns was 102, 26 and 17 days respectively. Annual survival probability for singletons, twins and triplets was 0.384 ± SE 0.084, 0.305 ± SE 0.058 and 0.267 ± SE 0.226, respectively (Figure 2.6). Survival was significantly higher in singletons when compared to twins ($\chi_1^2 = 6.03$, $P = 0.037$) and triplets ($\chi_1^2 = 8.65$, $P = 0.009$). However, twin and triplet litter mates had similar survival probabilities ($\chi_1^2 = 2.24$, $P = 0.292$). Because survival was similar for twins and triplets and we had only 5 radio-collared triplets during the 3 capture years, we grouped fawns in to 2 groups as litter size =1 or litter size ≥ 2 for multivariate modelling.

Birth weight ($\chi_1^2 = 2.81$, $P = 0.093$), Julian birth date ($\chi_1^2 = 1.66$, $P = 0.197$), age at capture ($\chi_1^2 = 0.31$, $P = 0.574$), chest girth ($\chi_1^2 = 1.73$, $P = 0.187$), total body length ($\chi_1^2 = 2.19$, $P = 0.138$), being born before or after average peak birth date ($\chi_1^2 = 1.36$, $P = 0.243$) did not contribute in predicting fawn survival probability on univariate analysis using Cox proportional hazard models from birth to 8 months.

2.4.4.2 Univariate effects of doe attributes on overall fawn survival up to 8 months

Overall annual fawn survival did not vary in association with any of the following variables: doe age group ($\chi_1^2 = 2.87$, $P = 0.089$), doe rBCS group by median ($\chi_1^2 = 0.07$, $P = 0.791$) or doe IFBF% group by median ($\chi_1^2 = 0.37$, $P = 0.542$).

We collared a total of 68 fawns (36 died, 32 censored) born to does not infected with CWD. We collared 16 fawns (11 died and 5 censored) born to CWD-infected does. We collared

fawns whose dam CWD status was inconclusive or unknown (uncollared does); these were not included in the analysis determining effects on fawn survival by dam CWD status. Median survival days for fawns of CWD negative dams was 54 days versus 42 days for fawns of CWD infected does. In univariate analysis, fawns of CWD negative females had a survival rate of 0.391± SE 0.066 for 8 months while fawns born to CWD infected dams had a survival rate of 0.286 ± SE 0.118 (Figure 2.7). Survival of fawns born to CWD positive dams was similar to survival of fawns born to CWD negative dams ($\chi_1^2 = 0.82$, $P = 0.364$).

2.4.4.3 Multivariate survival modelling from birth to 6 months

2.4.4.3.1 Overall survival from birth to 6 months

Using univariate analysis for overall survival data, litter size ($\chi_1^2 = 3.92$, $P = 0.047$), estimated birth weight ($\chi_1^2 = 2.81$, $P = 0.093$), Julian birth date ($\chi_1^2 = 1.66$, $P = 0.197$), chest girth ($\chi_1^2 = 1.73$, $P = 0.187$), total body length ($\chi_1^2 = 2.19$, $P = 0.138$), and radiocollar weight as percent of body mass by 2% group ($\chi_1^2 =$, $P = 0.071$) were selected as candidates for model building. As the litter size variable was non-proportional, we stratified the multivariable model according to two litter size strata. The best multivariate model included only the radio-collar: body mass ratio categorical variable, which was not significant in predicting overall survival of fawns up to 6 months ($\chi_1^2 = 3.40$, $P = 0.065$) (Table 2-7).

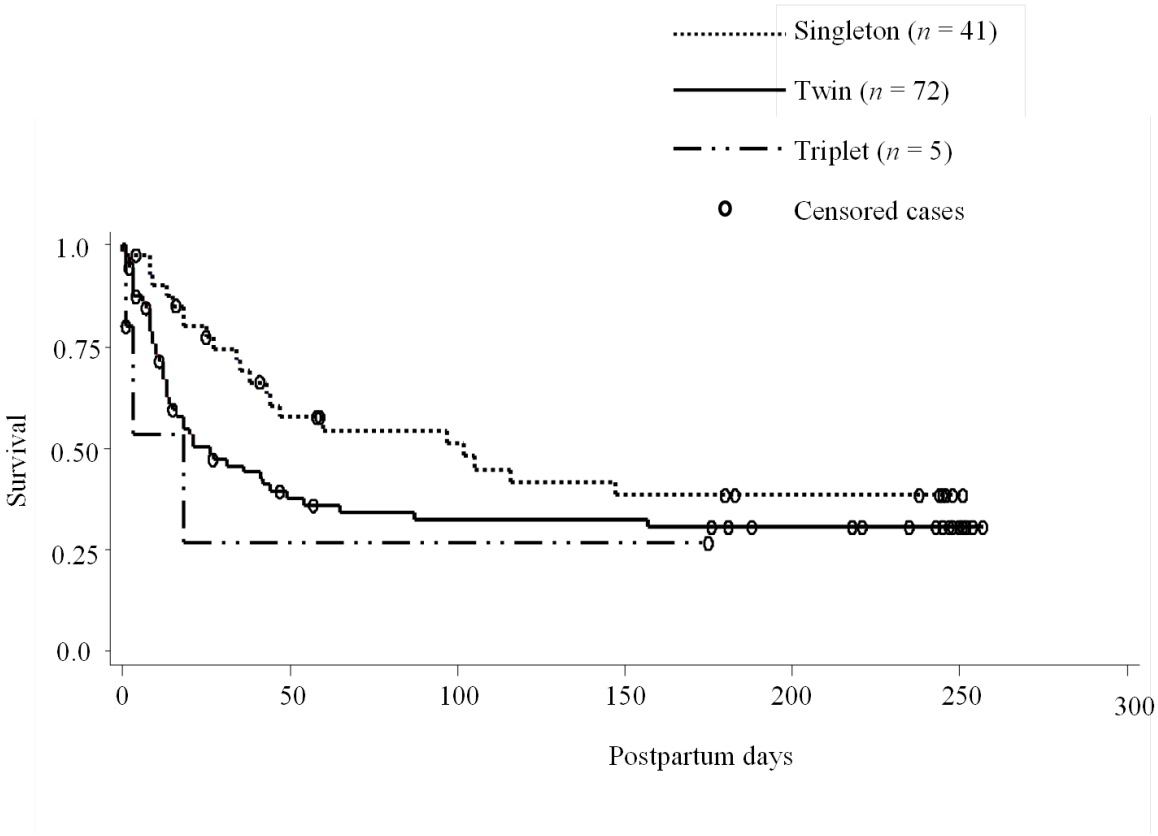

Figure 2-6: Survival curves for singleton, twin and triplet litters for mule deer neonates collared from 2009 to 2011.

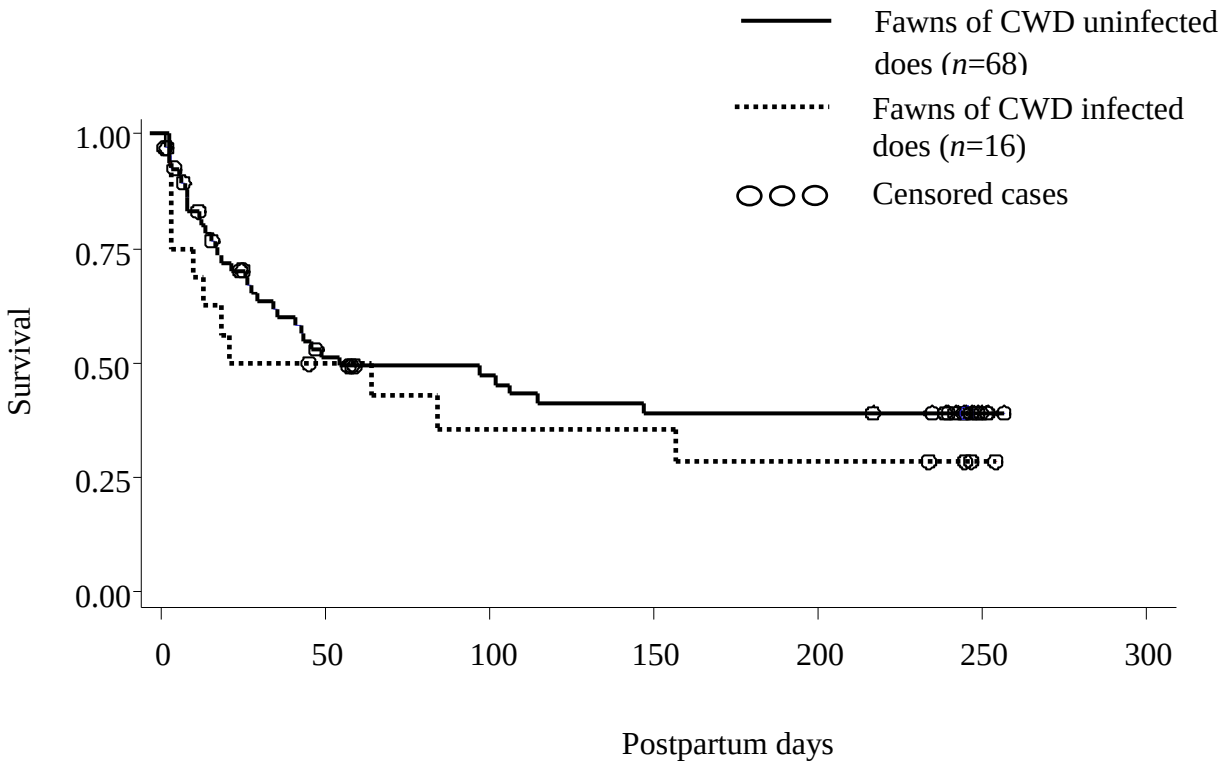

Figure 2-7: Survival curves for mule deer fawns born to CWD infected and CWD uninfected does in Antelope Creek from 2009 to 2011.

Table 2-7: Models explaining survival of mule deer fawns within 6 months postpartum in Antelope Creek from 2009 to 2011. Parameters considered include: estimated Julian birth date (1), chest girth (2), total length (3), estimated birth weight (4), and fawn group by radio-collar: body mass ratio ≥ 2 % (5). df= degrees of freedom

Model parameters	χ^2	df	*P* value	-2 Log L	AIC	*n*	AICc	ΔAICc	w_i
Global	6.04	5	0.301	469.405	479.405	114	479.96	5.878	0.019
1,3-5	6.04	4	0.195	469.407	477.407	114	477.77	3.692	0.056
1,4,5	5.72	3	0.125	469.724	475.724	114	475.94	1.860	0.140
1,5	4.81	2	0.090	470.636	474.636	114	474.74	0.662	0.254
5	3.40	1	0.065	472.046	474.046	114	474.08	0	0.354
null	-	0	-	475.452	475.452	114	475.45	1.370	0.178

2.4.4.3.2 Survival for 0 -7 day postpartum period

For the period within 1 week of birth, year ($\chi_1^2 = 6.85$, $P = 0.009$), litter size ($\chi_1^2 = 4.71$, $P = 0.030$), birth weight ($\chi_1^2 = 5.02$, $P = 0.025$), age at capture ($\chi_1^2 = 4.78$, $P = 0.029$), chest girth ($\chi_1^2 = 8.06$, $P = 0.004$), total length ($\chi_1^2 = 9.81$, $P = 0.002$) and radiocollar : body mass at capture by 2% group ($\chi_1^2 = 8.50$, $P = 0.003$) were selected as candidates for a multivariable model. All of these variables satisfied proportionality in the multivariate model. The model with the highest Akaike weight contained covariates: year 2011 with reference to year 2009, litter size ≥ 2 compared to litter size < 2, total length as well as fawn group by radiocollar: body mass ratio 2 % (Table 2-8). The variables: year 2011 and litter size were poor predictors of survival within 7 days ($P > 0.05$) (Table 2-9). Being a litter member of triplets increased risk of death by almost 7 fold (hazard ratio: 6.88, 95% CI: 0.87- 52.9), while being born in 2011 increased risk of death within 1 week of birth by almost 3 fold (hazard ratio: 2.86, 95% CI: 0.98-8.37) (Table 2-9). However, these hazard ratios were not significant. Having a 1 cm longer total length at birth reduced the risk of death by 8%; the hazard ratio of 0.93 for total length which was a significant effect (95% CI: 0.62-0.97) (Table 2-9). Furthermore, fawns having a higher radiocollar: body mass ratio than 2% were almost 4 times more prone to die within 7 days after birth (hazard ratio: 3.86, 95% CI: 1.27-11.6). None of the interaction terms between any of these 4 variables were significant in the final model ($P > 0.05$)

Table 2-8: Model selection for mule deer fawn survival within 7 days post-partum in Antelope Creek from 2009 to 2011. Parameters considered include 2010 with reference to 2009 (1), 2011 with reference to 2009 (2), twin/triplet litter size with reference to singleton group (3), chest girth (4), total length (5), birth weight (6), age at capture (7), radiocollar : body mass at capture by 2% group (8). df = degrees of freedom.

Model parameters	χ^2	df	*P* value	-2 LogL	AIC	AICc	ΔAICc	w_i
Global	26.46	8	0.0009	91.696	107.696	109.067	8.13	0.008
1-3, 5-8	26.45	7	0.0004	91.703	105.703	106.759	5.822	0.027
1-3,5,7,8	26.41	6	<0.0002	91.740	103.740	104.525	3.588	0.081
2,3,5,7,8	26.24	5	<0.0001	91.916	101.916	102.471	1.534	0.227
2,3,5,8	25.58	4	<0.0001	92.571	100.571	100.937	0	0.488
2,5,8	20.56	3	0.0001	97.598	103.598	103.816	2.879	0.116
5,8	16.92	2	0.0002	101.230	105.230	105.338	4.401	0.054
Null	-	0	-	118.158	118.158	118.158	17.221	0.000

Table 2-9: Final model explaining hazards within 7 days of birth for mule deer fawns in Antelope Creek from 2009 to 2011. 95% Confidence intervals (CI) for hazard ratios are given.

Model parameter	Estimate	SE	χ^2	*P* value	Hazard ratio	Lower CI	Upper CI
Year 2011 (with reference to 2009)	1.053	0.547	3.70	0.054	2.86	0.98	8.37
Litter size ≥2 (with reference to singletons)	1.929	1.050	3.37	0.066	6.88	0.87	52.9
Total length	-0.073	0.026	7.68	0.005	0.929	0.62	0.97
Radiocollar: body mass by 2% group	1.350	0.564	5.71	0.016	3.86	1.27	11.6

2.4.4.3.3 Survival for 8 – 30 day postpartum period

Only litter size (χ_1^2 = 4.82, P = 0.028), and birth weight (χ_1^2 = 2.07, P =0.149) were selected as candidates for a multivariable model from univariate survival analysis of all fawn and doe attributes for period of 8-30 days after birth. Litter size and birth weight satisfied proportionality in a multivariate model. From the global multivariate model we removed birth weight (χ_1^2 = 1.55, P = 0.212) to obtain the final model which included only litter size (Table 2-10). According to this model, being a member of a twin or triplet litter increased probability of death more than 2.5 fold compared to being a singleton (hazard ratio = 2.53, 95% CI: 1.14 – 5.57).

Table 2-10: Model selection for mule deer fawn survival within 8-30 days post-partum in Antelope Creek from 2009 to 2011. df = degrees of freedom

Model Parameters	χ^2	df	*P* value	-2 Log L	AIC	AICc	ΔAICc	w_i
Litter size, birth weight	7.58	2	0.022	285.658	289.658	289.765	0.525	0.404
Litter size	6.03	1	0.014	287.205	289.205	289.24	0	0.525
Null	-	0	-	293.242	293.242	293.242	4.002	0.071

Table 2-11: Model selection for mule deer fawn survival within 31-90 days post-partum in Antelope Creek from 2009 to 2011. Parameters in the models considered included sex (1), estimated Julian birth date (2), and group by peak birth date (3).

Model parameters	χ^2	df	*P* value	-2 Log L	AIC	*n*	AICc	ΔAICc	w_i
Global	3.65	3	0.301	69.517	75.517	92	75.789	3.824	0.070
1, 3	3.56	2	0.168	69.612	73.612	92	73.746	1.781	0.194
3	3.25	1	0.071	69.921	71.921	92	71.965	0	0.475
Null	-	0	-	73.173	73.173	92	73.173	1.207	0.259

2.4.4.3.4 Survival for 31-90 day postpartum period

For the 31-90 days postpartum period, out of the nominee variables selected from univariate analysis for multivariate Cox model, sex (χ_1^2 =0.305, *P* =0.580), and estimated Julian birth date (χ_1^2 =0.088, *P* =0.766) were removed from the global candidate model to obtain the final model which contained only fawn group by peak birth date (χ_1^2 =2.96, *P* =0.085, hazard ratio =3.35) as a variable, and possessed the highest Akaike weight(w_i) (Table 2-11). Even though, being born after June 12 (average peak birth date) increased risk of death by more than 3 fold within 1-3 months after birth (hazard ratio =3.35), fawn group by peak birth date was not significant in predicting 31-90 day fawn survival in the final model.

2.4.4.3.5 Survival for 91-180 day postpartum period

For the period of 91-180 days (3-6 months) after birth, litter size and group by doe BCS median were selected by univariate analysis as candidates for a multivariate Cox model. All these candidate variables behaved proportionally in the multivariate model. However; after removing doe BCS median (χ_1^2 = 0.00, *P* = 1.00) and litter size (χ_1^2 = 0.00, *P* = 0.99), the null model with no covariates was the best model predicting fawn survival between 91-180 days.

2.4.4.4 Radio collar effects on fawn survival

For all the fawns captured from 2009 to 2011, the ratio of radio collar mass: body mass at capture ranged from 0.5% to 2.9%. Sixteen (14%) of the 118 fawns radio-collared ratios ≥ 2%; all others were < 2%. Fawns with ratios of ≥ 2% and < 2% had statistically similar survival probabilities (χ_1^2 = 3.25, *P*= 0.071), indicating that there was no effect of radio-collar weight on overall fawn survival up to 8 months. However; when survival was further analysed, we found a significant, negative, univariate effect of having a radio collar: body mass at capture ≥ 2% on survival within 7 days of birth (χ_1^2 = 8.50, *P*= 0.003), but not for periods of 8-30 day (χ_1^2 = 0. 09,

P= 0. 757), 31-90 day (χ_1^2 = 0.01, *P*= 0.913) or 91-180 day (χ_1^2 = 0.74, *P* = 0.389) postpartum. Having a radio-collar that weighed more than 2% of body weight increased the risk of dying within 0-7 day period for a neonate 3.9 times compared to a lower ratio (hazard ratio= 3.86, 95% CI of hazard ratio: 1.4 – 11.6) (Table 2-8, Table 2-9).

2.4.4.5 Effects of handling on neonatal deaths within 4 days postpartum

In the pooled dataset, there were 22 fawns that died within 4 days of capture; 4 in 2009, 7 in 2010 and 11 in 2011. This included three sets of twins born to two CWD positive females in 2011 and to one CWD negative doe in 2010. Four of the dead fawns (2 in 2009, 1 in 2010 and 1 in 2011) were born to uncollared does while seven were born to yearling does. Three fawns (2 in 2010 and 1 in 2011) were found dead before collaring (Table 2-12). Fawns that were found dead and thus not handled alive, or fawns for which we had insufficient evidence to conclude it had died, were not included in this analysis. There was no difference between neonatal survivors and non-survivors at 4 days post-capture based on handling related attributes which included handling time (*Z* = -0.298 , *P* = 0.766) and age at capture (*Z* = -1.019 , *P* = 0.308). However, neonates that died within 4 days of capture had significantly lower mean ranks for estimated birth mass (*Z* = -2.295, *P* = 0.022), chest girth (*Z* = -2.346, *P* = 0.019) and total body length (*Z* = -2.345, *P* = 0.019) than those which survived beyond 4 days after capture and handling (Table 2-13).

Table 2-12. Summary of mule deer neonates that died within 4 days post-capture from capture years 2009 to 2011. Censored cases: three fawns which were found dead at capture and five fawns for which only a clean collar was found, are not shown. Age and CWD status was not available for uncollared does. n/a= data not available, ID = identification number.

Fawn ID	Capture year	Days survived after capture	Proximate cause of mortality	Doe ID	Doe age(years)	Doe CWD status
F27	2009	1	predation	Uncollared	n/a	n/a
F32	2009	0	abandonment	Uncollared	n/a	n/a
702	2010	1	unknown	155	7.7	negative
711	2010	1	predation	321	4.7	negative
746	2010	1	accident	Uncollared	n/a	n/a
818	2011	2	predation	437	1.7	negative
832	2011	1	predation	137	5.7	positive
833	2011	1	predation	137	5.7	positive
841	2011	4	abandonment	422	1.7	negative
848	2011	2	abandonment	623	1.7	negative
849	2011	2	accident	644	3.7	positive
853	2011	3	predation	507	3.7	negative
858	2011	0	predation	646	3.7	positive
898	2011	0	abandonment	465	1.7	inconclusive

Table 2-13. Comparison of birth attributes (birth mass, chest girth and total body length) and handling related attributes (handling time and age at capture) for mule deer neonates which died within 4 days of capture and which survived beyond 4 days. Fawns which were found dead initially, thus not handled or fawns for which death could not be confirmed were not included in the analysis.

Attribute	Mann-Whitney U statistic	*Z* statistic	*P* value
Estimated birth mass (kg)	366.5	-2.295	0.022
Chest girth (cm)	366.5	-2.346	0.019
Total body length (cm)	370.5	-2.345	0.019
Handling time (min)	505.5	-0.298	0.766
Age at capture (hours)	154.0	-1.019	0.308

Table 2-14: Summary of mule deer fawn recruitment (6-month-old, live fawns per female) from 2009 to 2011 in Antelope Creek. *n* = sample size of females. * Recruitment not calculated for CWD infected females in 2009 due to very low sample size.

	Capture year			All years
	2009	2010	2011	
CWD infected females:				
• fawns	1	2	1	4
• *n*	1	4	13	18
• recruitment	*	0.50	0.08	0.22
CWD uninfected females:				
• fawns	4	10	5	19
• *n*	12	28	20	60
• recruitment	0.33	0.36	0.25	0.32
Uncollared/CWD status inconslusive females:				
• fawns	6	0	1	7
n	0	1	5	6
Annual:				
• fawns	11	12	7	30
• *n*	13	33	38	84
• recruitment	0.85	0.36	0.18	0.36
• recruitment ratio(uninfected/infected)	0.33	0.72	3.12	1.45

2.4.4.6 Fawn recruitment

Recruitment for 2009, 2010 and 2011 were 0.85 (11fawns, 13 females), 0.36 (12/33), and 0.18(7/38) fawns per doe respectively (Table 2-14). There was a generalized reduction in annual recruitment from 2009 to 2011 for CWD uninfected as well as infected females. In 2011, of 13 CWD infected females alive in following winter, only 1 fawn was observed at heel which was a contrast to at least 2 fawns being alive for 4 CWD-infected females in 2010. In 2011, uninfected dams were on average 3 times more successful in fawn rearing up to Dec 12, compared to CWD infected does.

2.4.5 Cause specific mortality

From 2009 to 2011, the death of 74 fawns was recorded; 22 in 2009, 27 in 2010 and 25 in 2011. This includes 3 fawns found dead prior to radio-collaring, 2 in 2010 and 1 in 2011. We were able to obtain portions or intact carcasses of 28 fawns for necropsy within the three capture years: 5 in 2009; 8 in 2010; and 15 in 2011 (Table 2-15). In all years, the majority of known mortalities were caused by predation (Table 2-16). In 2011, we found 2 radio-collared fawns that died of infection; one had a brain abscess and one had bacterial infection secondary to a suspected golden eagle (*Aquila chrysaetos*) attack. *Arcanobacterium pyogenes* was isolated from the brain abscess. During the first 2 years of the study, we did not find any fawns that died due to infection.

Abandonment was the cause of mortality in four fawns in 2011; two were born to primiparous does and the others were twin litter mates who were born to a CWD-infected, 4 year-old doe. In 2009, the single abandoned fawn was born to an uncollared female with unknown age and CWD status.

One fawn drowned in each of 2010 and 2011. In 2009, one fawn was shot by a hunter when it was 5 months old and one fawn succumbed to vehicle collision. Four deaths in 2010 were

attributed to accidents: two neonates with multiple skull fractures and trauma found prior to radio-collaring, one that died of aspiration of mud and water in a deep crevice and one that drowned. In 2011, a single fawn which was presumed to have died due to hypothermia after a day of heavy rain (as determined by laboratory necropsy of the whole intact carcass by ruling out other possible causes) was included in accident/trauma category. The proportion of cases in which the cause of death was unknown was greater in 2009 (54%) and 2010 (59%) than in 2011 (16%).

Table 2-15: Summary of necropsies performed on mule deer fawn carcasses retrieved as portions or intact from 2009 to 2011

		Number of cases			
		2009	2010	2011	overall
Type of specimen	Portion of carcass	2	5	8	15
	Intact carcass	3	3	7	13
Final diagnosis on necropsy	Predation	2	4	7	13
	Abandonment/starvation	1	0	4	5
	Accident/trauma/hunting	1	3	1	5
	Infection	0	0	2	2
	Unknown	1	1	1	3

Table 2-16: Cause of mortality of mule deer fawns radio-collared or found-dead from 2009 to 2011 as determined from necropsy and field mortality site examination in Antelope Creek. *n* = number of total fawns examined, % = proportion of total fawns examined.

Cause of mortality	2009		2010		2011		Overall	
	n	%	*n*	%	*n*	%	*n*	%
Predation	6	27	7	26	14	56	27	36
Abandonment/starvation	1	5	0	0	4	16	5	7
Accident/trauma/hunting	3	14	4	15	1	4	8	11
Infection	0	0	0	0	2	8	2	3
Unknown	12	54	16	59	4	16	32	43
Total	22	100	27	100	25	100	74	100

Table 2-17: Weather parameters for Swift Current weather station for 2009/10 and 2010/11 with 30-year-average obtained from Environment Canada (2011).

Parameter	2009/10	2010/11	30 year average
Total days with minimum temperature < (-20°C)	31	51	32
Total days with snow depth > 10 cm	39	127	40
Winter severity index (WSI)	70	178	72
Total precipitation from May to October 31 (mm)	184	470	254

2.4.6 Weather Effects

The winter of 2010/11 was a harsher-than-average winter while the preceding winter was average. The winter of 2010/11 had three times as many days with a snow depth > 10 cm compared to 2009/10 and approximately twice as many days with temperatures < - 20°C (Table 2-17). The winter severity index (WSI) for 2009/10 was 70 and was similar to the 30-year average. For 2010/11, the calculated WSI was 178 and this was 2.5 times the 30-year average. There was considerably less precipitation in 2009/10 than average, while the next summer had a total precipitation of 470 mm, which was a record high for the area.

2.5 Discussion

For all yearling and older females captured in 2010 and 2011, the pregnancy rate was 100% with an average of 1.99 fetuses per doe. These pregnancy and fetal rates are higher than some reports in the literature for mule deer: 94.7% and 1.82 (Robinette et al. 1973), 95.2% and 1.72 (Salwasser et al. 1978), 93% and 1.70 (Andelt et al. 2004) but are similar to others: 100% pregnancy rate during first two years of deer study in Texas (Haskell 2007) and 98% in Arizona desert mule deer (Tatman 2009) but they had reported lower fetal rates, 1.8 and 1.77, respectively. We did not detect a difference between fertility of yearlings and older females, as has been reported previously for captive and free ranging deer (Robinette et al. 1973, Brewster 1993). However, because of small sample size of yearling does in our study, caution should be applied in interpretation of age specific reproductive trends.

Adult females in Antelope Creek had lower winter body condition in 2010/2011 than in 2009/2010. The winter of 2010/2011 was more severe, as evidenced by a winter severity index of 178, which was 2.5 times higher than that of 2009/2010 (WSI=70) and of the calculated 30 year normal value (WSI=72) (Environment Canada 2011). Winter weather is a major determinant of body condition and in turn the reproduction of cervids in temperate climates

(Runge and Wobeser 1975, Brewster 1993). We did not observe any noticeable effects of CWD infection status on any of the winter body condition indices. CWD has a prolonged but variable incubation period before progressing to a clinical stage; emaciation and weight loss are considered to be hall marks of the clinical phase but may not be apparent in subclinical stages (Williams and Young 1980, Williams 2005). Ultrasound scanning is considered to be of limited value in assessing deer in poor condition during winter and is unreliable in calculating IFBF % when rump fat thickness is less than 0.2 cm (Cook et al. 2007), as was the case in this study. However, ultrasound and palpation remain the most useful and widely used techniques in evaluating live animal body condition in wild deer (Cook et al. 2007, Bishop et al. 2009, Tollefson et al. 2010). Rump fat was difficult to detect by ultrasound scanning in 2011 and only 7 females had rump fat of ≥ 0.2 cm, compared to 19 females with measurable fat thickness in 2010. None of the dam body condition indices (rBCS, IFBF%, BRI and LIVINDEX) were important in predicting fawn survival in any of the postpartum periods. Some previous research had found that female body condition was weakly related to mule deer fawn survival (Johnstone-Yellin et al. 2009, Tatman 2009), although other studies reported that better dam body condition lead to improved survival of off-spring (Testa and Adams 1998, Keech et al. 2000, Lomas and Bender 2007). The small number of females with measurable fat thickness (n =26), as well as only 5/26 females having IFBF % > 6.9, may have significantly affected statistical power in our survival analysis.

During this study, pregnancy and fetal rates did not vary between years with differing winter severity. Similar reproductive rates were observed for white-tailed deer in southern Saskatchewan that did not face severe weather prior to conception (Brewster 1993). It is also possible that higher than usual precipitation in summer and early autumn of 2010 resulted in

females entering rut in better condition, which led to high conception rates that may have balanced the negative effects of severe winter weather during late pregnancy in 2010/2011. As energy required for maintaining lactation is comparatively higher than that of gestation, probably all females conceived while being hopeful for better spring nutrition for lactation. A recent study by Tollefson et al. (2010) reported that improved body condition at the end of fall as a consequence of higher nutrition in summer and early fall, may contribute considerably to reproductive performance in mule deer. The same authors suggested that lactating does that ingested poor quality vegetation after dry warm months and experienced early forage declines would enter breeding season and winter with reduced body energy reserves. Furthermore, we think that assessing female body condition one month later in 2011 compared to previous capture year also contributed to the poorer condition observed in 2010/2011.

Multiple factors likely biased our sample of fawns and their mean capture date in 2009, which was a season of opportunistic fawn capture without use of VITs. That year, we commenced the fawn capture period based on previous field observations and used doe behavior to find neonates (Carstensen et al. 2003). We terminated capture in the third week of June after reaching our target number of fawns. Furthermore, fewer radio-marked females were involved in our study in 2009. Implantation of VITs during the last two capture years helped us in determining the natural duration of fawning season for mule deer in the study area. Usage of VITs has been a recent developing trend in studies of neonatal deer, as these devices reduce time spent per fawn search and sampling bias, and increase capture rates of neonates within 0-2 days of birth (Bishop et al. 2007, Haskell et al. 2007, Johnstone-Yellin et al. 2009, Tatman 2009) .

Only 33% of fawns in the Antelope Creek study area survived to 8 months of age. This survival rate was lower than that observed for mule deer fawns in some locations: fawns

provided with a nutritional treatment (0.528) or control group (0.478) (Bishop et al. 2009); 0.501 in west-central Colorado (Pojar and Bowden 2004); fawns of successful VIT-does (0.558) and no-VIT-implanted does (0.471) in south west Colorado (Bishop et al. 2007), and better than that in other locations: Tatman (2009) reported only 16% survival to 8 months in desert mule deer in Arizona and Bleich et al. (2006) reported less than 30% fawn survival for 3 out of 4 study years in California with an overall survival of 27.9% up to 6 months. We observed 5% and 10% loss of fawns within 7 days postpartum in 2009 and 2010, respectively, which is similar to the 7.6% fawn mortality within the first week of life reported by Robinette et al. (1973) for captive mule deer. In 2011, 26% of fawns died within 1 week of birth similar to a 22% loss observed in Texas (Haskell 2007). Whittaker and Lindzey (1999) reported a survival probability of 0.66 for mule deer neonates within 30 days of birth which was similar to fawn survival at Antelope Creek during 8-30 day period (0.634 ± SE 0.049). In our study, the lowest survival rate occurred within a month of birth, and overall survival trends indicated that fawns which survived the first month of life were likely to survive the first winter. Comparable fawn survival tendencies have been documented for mule deer elsewhere (Pojar and Bowden 2004, Johnstone-Yellin et al. 2009).

Early fawn survival was positively associated with total body length. For the 0-7 day postpartum period, the hazard ratio for total body length was 0.929 (95% CI 0.62 - 0.97) indicating a 7.1% lower risk of death for fawns with 1 cm larger body length. Tatman (2009) documented a similar positive relationship between neonatal body length and survival for mule deer during the first 4 weeks of life. Although litter size was not a good predictor of 0-7 day survival, it was the best predictor of survival in the 8-30 day period. Twin or triplet litter mates had 2.5 times higher risk of dying in comparison to singletons (hazard ratio=2.53, 95% CI: 1.14 – 5.57). Robinette et al. (1973) reported higher mortality rates for twins (4%) and triplets (17%)

than singletons (0%) within 7 days after birth for multiparous captive mule deer does. Having litter mates predisposes young mule deer and other cervids to a higher risk of death (Linnell and Anderson 1998, Johnstone-Yellin et al. 2009). However, it is less likely that mule deer siblings were dependent on each other for survival as reported in recent neonatal mule deer studies (Bishop et al. 2008, Johnstone-Yellin et al. 2009). Bishop et al. (2008) found that littermate fates were independent of maternal characteristics and their risk of predation was also independent. Independence in terms of being predated could be as a result of the behavior of does to separately locate neonatal offspring and characteristic individual hider behavior displayed within first few weeks after birth. Our results are consistent with other authors who have reported a negative correlation between fawn survival rate and parturition date within the fawning season (Lomas and Bender 2007, Tatman 2009). Having a birth date later than the mean birth date was negatively related to fawn survival during the 31-90 day period because being born 1 day later increased risk of dying by 18% (hazard ratio: 1.18, 95% CI: 1.05-1.32). Older age of females and precipitation during pre-rut and rut periods could lead to earlier birth dates while deer living in overgrazed geographical locations have later parturition dates (Haskell 2007).

Overall birth rate was 1.29 fawns per doe for pooled data in Antelope Creek. The overall birth rate was higher in 2009 (1.48 fawns per doe) but similar between last two capture years. Annual birth rates apparently decreased consistently but to a slower degree from 2010 to 2011 than from 2009 to 2010, irrespective of doe CWD infection status. Overall birth rate dropped to 1.13 fawns per doe for infected females in 2011 from 1.6 fawns per doe in 2010. A negative trend similar to what was seen for birth rate among years was observed when annual fawn recruitment was calculated at 6 months of age. In 2010, recruitment for infected females was 0.5 fawns per female and it decreased to 0.08 fawns per doe in 2011. This lower recruitment for

infected females was further evident when recruitment ratio for uninfected females was compared with infected females. In 2010, for 28 uninfected females, fawn recruitment was 0.72 times less compared to recruitment for 4 infected females the same year. One year later, in 2011, uninfected females were apparently 3.12 times more successful in raising fawns up to 6 months than infected females while recruitment for uninfected females dropped slightly from 0.36 to 0.25 fawns per doe.

Very few studies have explored effects of CWD on fawn production and recruitment in wild deer populations (Dulberger et al. 2010, Blanchong et al. 2012). In south-central Wisconsin, subclinical CWD infection had no effect on offspring production of hunter harvested white-tailed deer (Blanchong et al. 2012). This deer sample obtained from hunter harvest may have been biased in age composition, CWD infection status and other factors and make it difficult to compare with our results. Dulberger et al. (2010) reported that mean recruitment ratio at 2-2.5 months of fawn age between CWD uninfected to infected mule deer was 1.5 per year in Boulder, Colorado where CWD prevalence was about 28%. Furthermore, the same authors concluded that the effect of increasing CWD prevalence on adult female survival and recruitment taken together had the most negative effect on population growth, while the effect of CWD on recruitment alone had trivial effects on population growth. In our study, we assessed fawn recruitment when fawns were 6 months of age. Although most deaths in Antelope Creek occurred within 3 months of age, additional fawn deaths occurred between 3 and 6 months after birth but not between 6 and 8 months of age during all 3 capture years. Thus, it was more accurate to calculate annual fawn recruitment at 6 months of fawn age. As expected, our recruitment rates for CWD infected (0.22 fawns/doe) and uninfected (0.32 fawns/doe) dams were lower than that reported for

infected females 0.95 fawns/doe and 1.34 fawns/doe for uninfected females by Dulberger et al. (2010).

Other TSEs are known to cause reproductive consequences on host species. Scrapie infected sheep had lower offspring production, while specific genotypes had lower lifetime breeding performance in the United Kingdom (Chase-Topping et al. 2005). Bradley (1991) reported that infection with bovine spongiform encephalopathy may lead to negative effects on lactation in parturient cows. Statistical analyses on recruitment data that were not conducted in this study may provide clearer trends over the years in Antelope Creek.

In our study, there was no relationship between dam subclinical CWD infection status and fawn survival up to 8 months or any other period. Although, subclinical CWD infection status did not seem to have any direct effect on fawn survival, other indirect effects, that were not considered in this analysis, but related to being infected, could bring about behavioral changes that may compromise offspring survival. Most recently Blanchong et al. (2012) reported that male fawns born to subclinically CWD infected dams were more likely to be shot by hunters during the fall hunting season than that of uninfected dams. Same authors suggested that this may be due to reduced parental care provided by CWD infected mothers which may have resulted in less dam-dependent male fawns at an early age. Thus male fawns born to CWD infected dams could have been more susceptible to be harvested than fawns born to CWD uninfected females. Behavioral changes during the subclinical stages in the course of CWD which is less known, but are likely, include lack of awareness, responsiveness or even deficits in vision as explained for clinical stage of the disease (Williams and Young 1980, Williams 2005). In clinically affected mule deer, ruminal contents may contain sand and gravel (Williams and Young 1980). If dietary changes occur during the subclinical disease course and if those changes

are substantial enough to have quality and quantity of lactation in a subclinically CWD infected parturient deer, it may bring about disadvantageous consequences to growth and survival of offspring.

Mule deer commonly wean their fawns between 4 to 6 months of age (Anderson and Wallmo 1984, Gaillard et al. 2000) and, during the preweaning period, fawns are highly dependent on the dam for nutrition, as well as for safety from predators and other environmental hazards. During fawn searches in Antelope Creek, we observed reduced awareness and response in infected dams which included remaining in the area for a considerable time period despite human disturbance. In 2010, one infected female, remained within 20 m during processing of her twins and did not flee or respond to calls made by her fawns during processing. Her twins died due to predation and unknown causes within 84 and 64 days, respectively. In 2011, a female, who was not captured or implanted with a VIT as she was diagnosed with subclinical infection the previous year, did not flee on three occasions until fawn crews were 10-20 m away from the dam to carry out opportunistic fawn searches. Her twin fawns succumbed to predation between 10 and 13 days postpartum. Thus, neurological and behavioral effects of CWD on fawn bearing dams might cause decelerating effects on survival of newborns during their most defenseless life stage.

The most common known cause of mortality among mule deer fawns in Antelope Creek was predation, as has been documented in other locations (Linnell et al. 1995, Bishop et al. 2009, Johnstone-Yellin et al. 2009, Tatman 2009). However, disease (Myers 2001, Pojar and Bowden 2004) or starvation/abandonment (Lomas and Bender 2007) have been identified as the leading cause of mortality in different environments and populations. In 2011, when the study area received comparatively less spring and summer precipitation, we observed a higher proportion of

mortality as a result of predation (56%, 14/25) and starvation/abandonment (16%, 4/25) than either 2009 or 2010. Haskell (2007) reported increased fawn fatalities because of predation and sickness/starvation coincident with drier weather in west-central Texas. However, unknown causes of mortality accounted for more than half of total mortalities in 2009 (54%) and 2010 (59%). These unknown mortalities undoubtedly carried mortality events that occurred due to the known causes: predation, starvation/abandonment, trauma and disease. In all capture years, we were unable to determine the cause of mortality to differing degree, although we typically reached a fawn mortality within 24 hours of mortality signal detection in all capture years. The radio-collar switched to mortality signal only after being immobile for at least 4 hours, so that when predators or scavengers moved the carcass or radio-collar, which was often the case, this delayed emission of a mortality signal and hindered retrieval of whole or portions of carcass for examination.

Several factors may explain why the proportion of unknown cause of mortalities was higher in the first two capture years than in 2011. In 2009, a limited number of personnel were involved, and lack of experience probably contributed to inefficiencies in gaining maximum amounts of mortality site data crucial for determining a proper cause of death. In 2010 spring and summer, in spite of having more experienced personnel involved, the flooded and muddy landscape, as a result of unusually heavy rain, not only slowed searches and retrieval of mortalities, but may also have washed crucial pieces of mortality site evidence away, often leaving only a chewed radio-collar and bone fragments. In 2011 spring and summer, comparatively dry weather drastically improved detection and access to mortality sites within a minimum time and improved collection of essential mortality site data. We were able to gather 7

intact carcasses, as well as 8 portions in 2011 for laboratory necropsy, which is twice as many obtained in either of the two previous capture years.

We determined that 7% (5/74) of mortalities were caused by starvation, likely as a result of abandonment, including one case in 2009 and four cases in 2011. Livezey (1990) documented that marking induced neonate abandonment was underestimated in ungulate studies and listed several predisposing factors including interruption during the imprinting period, doe age, and nutritional status of dam. In our study, half of the abandoned fawns in 2011 were born to primiparous does. It is not uncommon for younger does with less reproductive experience, to be less able to relocate their fawns after disturbance by predators or humans, ultimately resulting in reduced fawn care (Ozoga and Verme 1986). We did not search for fawns at least 5 hours after VIT expulsion to facilitate maximum fawn-dam bonding. Other researchers have suggested that disturbance occurring 2 hours after birth, results in trivial risk (Bishop et al. 2007, Haskell 2007). However, Livezey (1990) reviewed natural abandonment as a function of age of dam, condition of dam and offspring, as well as population density and found that marking induced abandonment could be a result of disturbance at imprinting period, confusion of olfactory and visual recognition by plastic gloves and transmitters, and capture related stresses such as long-distance pursuits. During 2010 and 2011, we were able to reduce disturbance on imprinting and bonding after birth, as we could determine post-partum time elapsed with the use of VITs. In 2009 we did not utilize VITs in aid of fawn capture and thus did not know how much time had elapsed after a birthing event. We believe that the single fawn abandoned in 2009 was reached too early at the birth site as it was wet and covered with fetal membranes when found initially, and it was found dead on the following day with an empty abomasum. In 2010, a singleton born to a dam with a non-functional VIT was reached within < 1 hour of birth, the neonate was wet

and covered with fetal membranes when found, but it was not abandoned by 5-year-old dam and lived for 3.5 months. Haskell (2007) concluded that possibility of marking related abandonment was insignificant for sympatric mule and white-tailed deer in west-central Texas. The same authors concluded that it is difficult to determine or separate natural or marking induced abandonment due to the combination of contributing factors which are almost impractical to examine individually in a wild situation.

We found no difference in handling time and age at capture between fawns that succumbed within 4 days of capture and those that survived. Thus, we could not detect a relationship between human processing at capture to neonatal deaths within 4 days after capture. We noted considerably lower estimated birth weight, as well as body condition parameters (i.e.: chest girth, total body length) in fawns that did not survive 4 days post-capture. However, birth weight and chest girth length had no effect on fawn survival. Total body length was a good predictor of fawn survival within 0-7 days postpartum and undoubtedly having smaller body lengths at birth lead to higher risk of early death. Handling time and age at capture were not related to fawn survival up to 8 months in the pooled data set, 0-7 day, 8-30 day, 31-90 day or 91-180 day periods. These findings suggest that non-survivors were born weak and were prone to be naturally abandoned resulting in starvation and death than survivors within 4 days postpartum.

According to the Canadian Council on Animal Care (2003) – Guideline 28: on the care and use of wildlife "telemetry devices should weigh less than 5% of the body mass of animal involved". The maximum radio collar: body mass ratio for mule deer fawns in this study was 2.9% which was well within the guidelines. However, we found that radio-collaring was associated with an increased risk of death, within 7 days of capture, for fawns that had a low capture weight and a radio-collar: body mass ratio $\geq$ 2% when compared with fawns with higher

capture weights and lower radio-collar: body mass ratio. This is consistent with the findings of Skelton (2010), who reported similar adverse effects of radio-collars on juvenile mule deer survival, even though the collar: body mass ratio was < 5% and suggested that the cut-off limit should be reduced to 2%. Given these findings, the guidelines for maximum radio collar: body mass ratio should be reduced to 2%, at least for ungulates.

In this study we estimated female reproductive performance and fawn survival in a wild mule deer population in southern Saskatchewan. We have shown, although fawn production was high in spring, only one third of them survived to 8 months of life. These research findings combined with other age-sex class survival estimates from other parallel studies in this area, will be used to estimate long term effects of CWD in wild mule deer. Our findings suggest that, in the absence of strategies to reduce CWD transmission, population declines will occur if low fawn survival and increasing adult deer losses due to CWD persists.

CHAPTER 3

EXPOSURE OF YOUNG MULE DEER TO INFECTIOUS AGENTS IN A CHRONIC WASTING DISEASE ENDEMIC AREA IN SOUTHERN SASKATCHEWAN

3.1 Abstract

Free-ranging deer share several infectious agents with domestic livestock. Gaining a complete understanding of the epidemiology and ecology of these agents requires knowledge of their behavior in all infected species. As a part of an ongoing project to study geographic spread and long term population effects of chronic wasting disease (CWD), we captured juvenile deer in winter from 2007 to 2011 in a CWD endemic area in southern Saskatchewan and performed various laboratory tests on fecal, blood and tonsil samples to detect either prevalence or seroprevalence to parasites, various viruses and CWD. Fecal analysis revealed prevalences of 86% (80/93) for *Orthostrongylus* sp., 2% (2/93) for *Nematodirus* sp., 6% (6/93) for *Trichuris* sp, 1% (1/93) for Trichostrongyloidea, 1% (1/93) for *Skrjabanema* sp, 29%(27/93) for *Moniezia* sp, 29% (27/93) for *Thysanasoma* sp., 10% (9/93) for *Eimeria* sp., 3% (3/89) for *Giardia* sp., 1% (1/89) for *Cryptosporidium* sp. and 0% (0/78) for flukes. Overall seroprevalence for bovine herpes virus-1(BHV-1), bovine viral diarrhoea virus (BVDV), parainfluenza 3virus (PI3) and *Neospora caninum* were 3% (3/92), 20% (19/93), 22% (20/93), and 3% (3/91) respectively. Prevalence for mule deer-LHV (mule deer-LHV) was 15% (13/85) for pooled data. Differences in prevalence were observed among years for *Orthostrongylus*, BVDV, PI3 and mule deer-LHV but not among sexes. In 2010 and 2011, we detected subclinical CWD infection in 2 and 1 juveniles, respectively, with an overall prevalence of 3% (3/95). Furthermore we collected blood from neonates in spring 2009 to 2011, inclusive, in order to assess the prevalence of BVDV infection by polymerase chain reaction and the seroprevalence to *N. caninum.* Out of a total of

85, only a single neonate had detectable BVDV in peripheral blood lymphocytes and 12% (13/106) were positive for *N. caninum* antibody titers. In addition, we noticed that all dams which were seropositive for *Neospora* gave birth to at least a single fawn. Due to the long incubation period of CWD even a low prevalence in juvenile deer suggests high infection pressure in resident deer. Annual variation in exposure to *Orthostrongylus* sp., BVDV, and PI3 indicate various factors such as weather and infection in livestock may affect prevalence.

3.2 Introduction

Wildlife share many infectious agents with humans and domestic species. Unraveling the vital role played by variety of wildlife species in the transmission of infectious agents is essential in order to fully appreciate the epidemiology and ecology of such agents, especially those which cause disease at the wildlife-livestock interface (Siembieda et al. 2011). Thus, multiple studies have reported varying degrees of exposure to multiple infectious agents in wildlife, specially focusing on currently known pathogenic organisms (Sadi et al. 1991, Aguirre et al. 1995, Rickard et al. 1999, Frolich et al. 2006).

Even though the majority of studies look at adult wildlife, young animals may be more susceptible to certain infectious agents due to their immunological hyporesponsiveness probably as a combined result of negative effects posed on self-immunity by maternal antibodies, insufficient exposure to develop an active immunity during a short period of time as well as incompetent immunological mechanisms itself (Colditz et al. 1996). Rickard et al. (1999) found that juvenile white-tailed deer were shedding more *Cryptosporiduim* oocysts and *Giardia* cysts than adults. Forrester (1992) reported higher prevalence and five times higher worm burden for *Haemonchus* spp. in white-tailed deer fawns than adult deer. Reports on intensity and yearly trends of infectious agent exposure are not common for early life stages in deer, especially in

Canadian prairies resulting in a knowledge gap in studying ungulate population and disease dynamics.

Chronic wasting disease (CWD) is a fatal infectious prion disease in deer, elk and moose which is predominantly found in North America (Williams and Young 1980, Williams 2005, Baeten et al. 2007). It is characterized by a slow but progressive course of neurological disease similar to other prion diseases such as scrapie in sheep, bovine spongiform encephalopathy-BSE in cattle, Creutzfelt-Jacob disease and kuru in humans, and mink encephalopathy in mink. CWD spreads primarily from direct or indirect contact of an infected animal or material in the environment (Miller and Williams 2003). Transmission from dam to offspring prenatally is thought to be a rare possibility but little is known of its significance in disease spread (Miller and Williams 2003, Williams 2005). Possibility of infection in fawns has been demonstrated for mule deer experimentally (Sigurdson et al. 1999). Grear et al. (2006) reported free-ranging juvenile white-tailed deer subclinically infected with CWD during hunter submitted deer surveillance. It is unlikely for clinical signs to occur in fawns in the light of a long incubation period which could be a minimum of 16 months (Williams 2005).

As a part of an ongoing project to study geographic spread and long term population effects of CWD, we captured juveniles of the year during winters from 2007 to 2011 and collected blood, tonsil and fecal samples. We also obtained blood from neonates captured and radio-collared in each spring from 2009 to 2011 for a parallel fawn survival study. Furthermore we analysed adult female serum from 2009 -2011 for antibodies for *Neospora caninum,* to compare that with neonates. Our primary objective was to estimate extent and patterns of exposure to selected infectious agents over a 5 year period in 8-month old juveniles and neonates over 3 years in the study area using different laboratory techniques.

3.3 Methods

3.3.1 Study area

Refer to section 2.3.1 for description of study area.

3.3.2 Juveniles

During late winter (February or March) of capture years from 2007 to 2011, juvenile mule deer of approximately 8 months of age were captured. This included neonates radio-collared in the previous spring (for 2010 and 2011) as well as newly captured juveniles of the year (all capture years).

3.3.2.1 Capture

Capture procedure was the same as described in section 2.3.2.1.

3.3.2.2 Sample collection

Blood samples were collected from jugular vein in to 4 tubes per juvenile deer: two ethylenedinitrilo tetraacetic acid, disodium salt (EDTA) (Vacutainer®, Becton Dickinson Labware, CA) tubes to separate buffy coat and two plain vacutainer (Vacutainer®, Becton Dickinson Labware, CA) tubes to separate serum. Samples were either separated into blood components at the field station right after collection or maintained at 4°C until further processing was done later on the same day. All tubes were centrifuged at 200 g for 10 minutes for separation of blood components in a centrifuge. Serum was separated after letting plain tubes stand at least an hour before spinning. Serum, plasma, and buffy coat were transferred in to labelled cryovials.

Fecal samples were collected directly from the rectum from anaesthetised deer in to sterile sample bags (VWR®, Ontario, Canada). Tonsillar biopsies were obtained in to 1.5 ml micro-centrifuge vials using a mouth gag, laryngoscope and 30 cm biopsy forceps. All samples were stored in -20°C until laboratory testing was performed.

3.3.3 Neonates

3.3.3.1 Capture

Refer to section 2.3.3.1 for description of neonate capture. Neonates were captured from 2009 to 2011.

3.3.3.2 Sample collection

Blood samples were collected from the cephalic vein or jugular vein in to 2 tubes per new born fawn, an ethylenedinitrilo tetraacetic acid, disodium salt (EDTA) (Vacutainer®, Becton Dickinson Labware, CA) tube to separate buffy coat and a plain vacutainer (Vacutainer®, Becton Dickinson Labware, CA) to separate serum. On occasions, where less than 1 ml of whole blood could be collected total volume was collected in the serum separator tube.

3.3.4 Diagnostic tests

The following diagnostic tests were performed for collected samples. Diagnostic tests for juvenile samples collected in 2007 and 2008 were performed and results were kindly provided by C. Fernando. We compiled all data from 2007 to 2011 to evaluate and understand annual trends in prevalence for different infectious agents.

3.3.4.1 Fecal flotation

Wisconsin double centrifuge technique was used in detection of gastrointestinal parasitic eggs and oocysts in mule deer feces (Cox and Todd 1962). Two paper cups (labeled A and B) (Georgia-Pacific Corporation, GA, USA) were used for each fecal sample. Five grams of sample was weighed in to cup A. Then 12 ml of water was added in to each sample and was left to soak for 5-10 minutes. Using a disposable wooden tongue depressor, a homogenous fecal mixture was prepared. After pulling a single layer of 20 cm x 20 cm cheese cloth on cup B, contents of cup A was poured in to cup B through the cheese cloth. Left-over contents of cup A was rinsed with additional 3 ml of water and was filtered in to cup B. Filtrate in cup B was then poured in to a

labelled 16 mm x 125 mm (VWR® International, PA, USA) glass test tube. Filtrate in a test tube was centrifuged for 10 minutes at 200 × g (Damon/IEC DIVISION, IEC Model HN-S centrifuge, Thermo Fisher Scientific, MA, USA). After that, supernatant was discarded while not disturbing the sediment by a single pouring motion. 5 ml of concentrated sugar solution (454 grams of table sugar, 6 ml of 40% formaldehyde, 355 ml water) was added and mixed with sediment using a mini-vortex mixer. Then, test tube was filled with the same sugar solution up to approximately 5 mm from the top. Drops of more sugar solution were added carefully using a dropper bottle until a slightly convex meniscus was formed on the top of the tube. A 22 mm x 22 mm glass coverslip (VWR® International, PA, USA) was placed on the meniscus carefully, avoiding any air bubbles getting trapped underneath the coverslip. Any large air bubbles on the meniscus were removed using the edge of the coverslip, before placing the coverslip on the meniscus. Test tube with coverslip was centrifuged for 10 minutes at 200 × g. Then, the coverslip was lifted on a plane vertical to the tube and was placed on a labelled glass slide (Surgipath, IL, USA). Finally, glass slide was examined under a light microscope (Nikon labophot-2, Nikon Corporation, Japan) under 10 x 10 magnification for gastrointestinal parasitic eggs and oocysts based on morphology (Foreyt and Foreyt 2001). Results were recorded as eggs/oocysts per gram of feces.

3.3.4.2 Baermann technique

Modified beaker Baermann technique was used to recover parasitic larvae from deer feces (Forrester and Lankester 1997, Jenkins et al. 2005). For each sample, an envelope was prepared by placing a tissue paper (Kimwipes®, Kimberley-Clarke Corporation, ON, Canada) between two layers of vinyl window screening meshes with 2 mm x 2 mm pores. Five grams of weighed feces was placed on the tissue layer in an envelope. Each envelope was placed carefully close to water surface of a 250 ml glass beaker filled with 200 ml of tap water horizontally, letting it to submerge completely while having the tissue paper beneath the sample. Care was taken that

envelope did not sink to the bottom of beaker. Beaker with envelope was left undisturbed at least 18-24 hours. Then, envelope was removed and discarded without disturbing the sediment at the bottom of the beaker. After letting the beaker stand for an additional 5 minutes in order for sediment to further settle, the beaker content was carefully aspirated using suction without disturbing the sediment. The remaining sediment (approximately 30 ml) was poured in to 2 labelled 16 mm ×125 mm glass tubes and centrifuged for 10 minutes at 200 × g (Damon IEC Model HN-S, Thermo Fisher Scientific, MA, USA). Being careful not to disturb the sediment, supernatant was syphoned at the meniscus until 2 ml of sediment was remaining. Combined contents of both tubes were poured to a petri dish. Under a dissecting microscope (Wild Leitz Canada Limited, ON, Canada), parasitic larvae were identified using morphological characteristics as described by Foreyt and Foreyt (2001) at 40 x magnification. Total number of larvae per gram of feces was recorded for each sample.

3.3.4.3 Sucrose Gradient and Fluorescent antibody test (FAT)

At least 1g of feces from each sample was used to test for cysts of *Giardia* species and oocysts of *Cryptosporidium* species from fluorescent antibody test using Aqua-Glo G/C Direct Comprehensive Kit (Waterborne^{TM}, LA, USA). About 8 ml of 0.1M phosphate buffered saline (PBS) was added to each weighed fecal sample in a paper cup. The sample was thoroughly mixed to form a slurry and then poured through a funnel lined by 2 layers of cheese cloth into a 15ml centrifuge tube (tube A). Methylene blue dyed sucrose solution (5 ml, specific gravity =1.13) was added to another 15 ml centrifuge tube (tube B). The filtered fecal slurry in tube A was added on top of the sucrose solution carefully along the wall of the tube B using a disposable plastic transfer pipette. Tube B with layered sucrose solution and fecal slurry was centrifuged for 5 minutes at 200 × g (Damon IEC Model HN-S, Thermo Fisher Scientific, MA, USA). Then, all the material above the sucrose solution was transferred to tube A from tube B including any

pellets formed at sucrose-feces interface and contents of tube A was centrifuged for 5 minutes 200 × g. After discarding supernatant, sediment was suspended in 1 ml of PBS and transferred to a 2 ml labelled cryovial (VWR® International, PA, USA). Fecal suspension (15 µl) was spread evenly on two 15 mm diameter wells of a fluorescent microscope slide (Esco® Technologies, PA, USA). After allowing it to air dry for 30 minutes, each slide was flooded with acetone and was allowed to dry. Diluted Crypt-a-Glo™ antibody (Waterborne™, LA,USA) and Giard-o-Glo™ antibody (Waterborne™, LA,USA) in a fluorescent antibody test kit was added to each well and the slide was incubated at 37°C for 45 minutes. After adding a drop of No-Fade™ mounting medium (Waterborne™, LA,USA) to each well and covering with a coverslip, samples were examined with a fluorescent microscope (Model Nikon Labophot-2, Nikon Corporation, Japan) at 100 × magnification. A sample containing *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts supplied with the kit was used as a positive control. Positivity or negativity was recorded for each sample regarding presence of *Giardia* cysts and *Cryptosporidium* oocysts.

3.3.4.4 Fluke test

A fecal sedimentation technique which utilizes Flukefinder® apparatus (Flukefinder®, ID, USA) with top and bottom unit (each with a fine wire screen at the bottom) was used to test for presence of fluke eggs in mule deer feces. First, about 2 grams of fecal pellets were weighed and mixed with 30 ml of tap water in a paper cup. This mixture was then poured on to the top screen of the Flukefinder® apparatus. The apparatus was then tapped gently to make the slurry pass through both screens before repeating this procedure 4-5 times for further fine filtration. The bottom unit was separated and inverted over a paper cup and debris and contents were backwashed from the screen in to the cup with a strong stream of tap water. The suspension was poured in to a 16mm x 125mm glass tube and was allowed to settle for 5 minutes. Carefully with a single pour motion, supernatant was discarded without disturbing the sediment. Then the

sediment was suspended with 8 ml of tap water and was allowed to stand for 2-3 minutes. After repeating this resuspension procedure 3 times, supernatant was swirled, poured into a petri dish and examined for fluke eggs with a dissecting microscope (Wild Leitz Canada Ltd, ON, Canada) at 25 × magnification.

3.3.4.5 Serum neutralization test

Serum neutralization tests were used to detect antibodies to bovine viral diarrhoea virus type-1(BVDV) and parainfluenza3 (PI3) virus by Prairie Diagnostic Services Inc., SK, Canada. Eight, three-fold serial dilutions were prepared from serum which had been previously heat inactivated at 56°C for 30 min. Each dilution of serum was inoculated in to Dulbecco's Modified Eagle Medium (Invitrogen Canada Inc., ON, Canada) which was then pipetted in duplicate into a 96-well tissue culture plate (BD Falcon^{TM}, Becton Dickinson Labware, CA, USA) together with positive and negative control sera. Dulbecco's Modified Eagle Medium (DMEM) contained 5% fetal bovine serum (Invitrogen Canada Inc., ON, Canada) and antibiotics: 100 IU/ml penicillin G (Invitrogen Canada Inc., ON, Canada), 100 µg/ml streptomycin (Invitrogen Corporation, Canada), 100 µg/ml gentamicin (Invitrogen Canada Inc., ON, Canada) and 1 µg/ml enrofloxacin (Invitrogen Canada Inc., ON, Canada). Either 100 µl of 50% tissue culture infective dose ($TCID_{50}$) of Singer strain BVDV-1 or SF-4 strain PI-3(American Type Culture Collection, VR-281, VA, USA) was added to each well. Each plate was then incubated for 2 hours at 37°C before adding embryonic bovine tracheal cells suspended in DMEM. Culture plates were incubated in 5% CO_2 with 85% humidity for 7 days at 37°C. Using an inverted microscope (Olympus CK-2, Baxter / Canlab Ltd., ON, Canada), the plates were examined for cytopathic effects. The reciprocal of serum dilution which inhibited viral cytopathic effects was considered the serum end-point titer. A serum sample was considered to have specific antibody if titers were ≥ 6 (Waldner 2005, Fernando 2010).

3.3.4.6 Competitive Enzyme Linked Immunosorbant Assay

3.3.4.6.1 Detection of antibodies for *Neospora caninum*

Neospora caninum antibody test kit (Catalog no. 280-5, VMRD Inc, WA, USA), a competitive enzyme-linked immunosorbent assay (cELISA), was used to detect exposure to *Neospora caninum* in 8-month old mule deer from 2007 to 2011. We also tested neonatal serum for evidence of passive transfer of *Neospora* specific immunoglobulin and serum of all pregnant does from 2009 to 2011 (collected in winter, approximately 3 months prior to neonate sample collection) to determine effects of *Neospora* infection on reproduction.

Tests were performed according to manufacturer guidelines. Briefly, 50 µl of serum samples as well as positive and negative controls supplied with the kit were transferred to an Antigen-Coated 96 Well Plate. The plate was tapped gently on the side to enhance coating of wells by sample before incubating for one hour at room temperature. The plate was then washed with diluted Wash Solution three times without letting the plate dry between washes. Diluted horseradish peroxidase labeled monoclonal Antibody Conjugate (50 µl) was pipetted in to each well. After making sure each well bottom was coated by the conjugate by gentle tapping, the loaded assay plate was incubated for another 20 minutes at room temperature. The washing procedure was performed and 50 µl of Substrate Solution was pipetted in to each well. After carefully tapping the side of the assay plate several times, it was incubated for 20 minutes at room temperature, in a dark place without direct sunlight. Finally 50 µl of Stop Solution was added to each well and the plate contents were mixed by gentle rocking and tapping before the absorbance was measured with a spectrophotometer set at 650 nm wave length. Percent inhibition (% inhibition) was calculated using the following formula:

% inhibition= 100- [(sample optical density × 100)/average Negative Control optical density]

For test validation, negative control was to produce an average optical density within the range 0.3 - 2.5. Average optical density of positive control was to produce an inhibition more than or equal to 30%. A sample was considered positive if percent inhibition was more than 30% while a negative sample was identified by an inhibition percentage lower than 30%.

3.3.4.6.2 Detection of antibodies for Bovine Herpes Virus -1 (BHV-1)

Infectious bovine rhinotracheitis virus gB Antibody Test kit (version 99-40262, Idexx Laboratories, ME, USA) was used on deer serum to detect exposure to bovine herpes virus -1 (BHV-1/Infectious Bovine Rhinotracheitis virus) within first 8 months after birth. The test was performed according to manufacturer guidelines. First, 50 µl diluted wash solution was pipetted in to each well before adding 50 µl of negative control, positive control and samples according to previously assigned wells on antigen-coated plate. With gentle tapping contents were mixed manually and tightly sealed plate was incubated for 2 hours at 37°C. After incubation, well contents were discarded and each well was washed five times with 300 µl of wash fluid. Then, 100 µl of IBR-gB specific monoclonal antibody horseradish peroxidase conjugate was pipetted in to each well followed by a repeated washing step as previously explained. TMB substrate solution (100 µl) was added to each well before incubating covered ELISA plate at room temperature for 10 minutes in dark location. Finally stop solution (100 µl) was added to each well and plate was read for absorbance at 450 nm after standardizing spectrophotometer on air. For the test to be valid, average optical density of negative control (OD_{NC}) has to be equal or higher than 0.75 and blocking percentage of positive control has to exceed 80%. The positivity or negativity of a sample was determined by the degree of blocking which was calculated by the following formula:

$$\text{Blocking percentage} = \frac{(OD_{NC} - \text{average optical density of sample})}{OD_{NC}} \times 100$$

If blocking percentage of a sample was < 45%, it was considered negative for IBR antibody titer. A positive sample was identified by a blocking percentage ≥55%. A sample was a suspect, if the blocking percentage fell between 45% and 55%.

3.3.4.7 Polymerase chain reaction

3.3.4.7.1 Conventional Polymerase Chain Reaction

Conventional polymerase chain reaction (PCR) was used to detect the presence of mule deer lymphotropic herpes virus (mule deer-LHV) in 8 month old mule deer. We used techniques developed by Fernando (2010) to amplify a 283 bp fragment of intergenic spacer region (ISR) between the glycoprotein B (gB) and DNA polymerase (DPOL) genes. Briefly, after extracting DNA from buffy coat a single step conventional PCR reaction was performed and the resulting product was confirmed by sequencing. To extract DNA from buffy coat, Qiagen DNeasy blood and tissue kit (Qiagen Inc.-Canada., ON, Canada) used. First 200 µl of buffy coat was added to 20 µl of proteinase K (600 mAU/ml, Qiagen Inc.-Canada., ON, Canada) in an autoclaved 1.5 ml microcentrifuge tube. After adjusting total volume to 220 µl with phosphate buffered saline, 200 µl of buffer AL which contains guanidine hydrochloride was added, mixed and incubated at 56 °C for 10 min. Then 200 µl of 98% ethanol was added to each tube and mixed well before pipetting the mixture into a DNeasy mini spin column (Qiagen Inc.-Canada., ON, Canada) placed in a 2 ml collection tube. The flow through was discarded after mini spin column was centrifuged at 6000 × g for 1 min and the column was placed in a new collection tube. After adding 500 µl of buffer AW1 which contains guanidine hydrochloride (Qiagen Inc.-Canada., ON, Canada) in to the column, each column in a collection tube was centrifuged for 1 min at

6000 × g. Each spin column was transferred to a new collection tube before adding 500 µl of buffer AW2 (Qiagen Inc.-Canada., ON, Canada) and centrifugation at 20 000 × g for 3 min. Then each spin column was removed carefully to prevent any contact with the final flow through and was transferred to an autoclaved, labeled 1.5 ml microcentrifuge tube. 100 µl of buffer AE solution (Qiagen Inc.-Canada., ON, Canada) was added to each spin column and centrifuged at 8000 g for 1 min for elution of nuclear material collected in spin column. Each spin column was centrifuged for 1 more minute at the same speed to improve gain of total DNA. Finally using a spectrophotometer (Nanodrop® ND 1000, ThermoScientific, NC, USA), content of DNA extracted was measured and the tubes were frozen in -20 °C until further laboratory testing was carried out.

The conventional PCR master mix per sample contained 5 µl of 10× PCR buffer (0.2M tris-HCl, pH 8.4), 0.5 M KCl), 2.5 µl of 50 mM $MgCl_2$, 2 µl each from primers 10pmol/µl LHVF1 and 10pmol/µl LHVR1(Fernando 2010), 250µM of each dNTP, 0.5 µl AccuStart taq polymerase (5U/ µl, Quanta Biosciences, Inc., MD, USA) and 2 µl of extracted genomic DNA from buffy coat. A previously confirmed positive sample (kindly provided by C. Fernando) was used as a positive control for each PCR procedure. PCR steps included incubation at 94 °C for 5 min, 40 cycles of denaturation (94 °C for 30 sec); annealing (60 °C for 30 sec); and extension (72 °C for 30 sec) followed by a final extension step at 72 °C for 10 min. Finally 5 µl of each PCR product was loaded in to wells in a 1% (w/v) agarose gel stained with ethidium bromide. Diluted GeneRuler DNA Ladder Mix of 100 bp DNA ladder was used as standard for DNA band size (Invitrogen Corporation, CA, USA).

For confirmation of positive samples obtained from conventional PCR, each sample was cycle sequenced at National research Council Canada Plant Biotechnology Institute, SK, Canada

using LHVF1 and LHVR1 primers. PreGap4 and Gap4 software packages were used to further process received raw sequence data (Staden Package, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK, http://staden.sourceforge.net/). Processed sequence data were then compared with ISR sequence between gB and DPOL genes (Fernando 2010) using blast search of online GenBank nucleotide database provided by National Center for Biotechnology Information (NCBI) (Altschul et al. 1990).

3.3.4.7.2 Real Time Polymerase Chain Reaction

Real time reverse transcription PCR was conducted by Prairie Diagnostic Services Inc., SK, Canada on buffy coat samples obtained from neonatal mule deer in order to check for new born fawns persistently infected with bovine viral diarrhoea virus.

BioSprint 96 One-For-All Vet Kit (Qiagen Inc.-Canada., ON, Canada) was used to extract viral RNA according to manufacturer's protocol. The master mix used per sample for the single step real time reverse transcription PCR procedure consisted of 12.5 µl of 2× RT-PCR buffer, 1 µl of 25× BVDV primer probe mix, 1 µl of 25× RT-PCR enzyme mix and 3 µl of template RNA from each sample. The reverse transcription step of the real time PCR was performed at 45 °C for 10 min and the initial denaturation step took 10 min at 95 °C followed by 40 amplification cycles of denaturation (95 °C for 15 sec) and annealing (60 °C for 45 sec) using a real-time thermal cycler (Stratagene-Mx3005P, Agilent Technologies, Inc., CA, USA). Reporter and quencher dyes used for BVDV were FAM and BHQ®-1 respectively for the TaqMan® probe. For validation of the test results a positive control should obtain a cycle threshold value less than 30 and no signal at cycle 40 for negative template control. A sample was considered test positive for BVDV antigens if a cycle threshold less than 38 was obtained.

3.3.4.8 Immunohistochemistry

Immunohistochemical staining for PrP^{res} was carried out by Prairie Diagnostic Services Inc., SK, Canada on tonsillar biopsies obtained from 8 month old deer in winter (Fernando 2010). First, 3 mm thick, trimmed tonsillar tissue sections in plastic tissue cassettes were fixed in 10% neutral buffered formalin for at least 24 hours. The tissues were then placed in 98% formic acid for 1 hour before being washed and replaced in 10% neutral buffered formalin for 16 hours. Tissue sections which were paraffin saturated in a tissue processor (Histomatic 266, Fisher Scientific, ON, Canada) were sliced in to 5 µm thin sections and mounted on glass slides before incubation at 45 °C for 1 hour. Slides were further incubated in a drying oven at 65 °C for 1 hour and then applied with EZ Prep™ solution (Ventana Medical Systems Inc., AZ, USA) to get rid of excess paraffin. Then Ventana tissue conditioner CC-1(Ventana Medical Systems Inc., AZ, USA) was used to recover tissue antigens before heating the slides at 95 °C for 8 min. Another incubation step was carried out for 82 min at 100 °C which included repeated application of Ventana tissue conditioner CC-1 solution every 4 min. After this, Ventana protease 3 (Ventana Medical Systems Inc., AZ, USA) solution was smeared on slides prior to incubation at 37 °C for 2 min. followed by a washing step with Ventana reaction buffer (Ventana Medical Systems Inc., AZ, USA). Then an indirect immunostaining technique was performed to identify infectious prion molecules in tonsillar tissues mounted on glass slides as follows. First, barcoded slides arranged in 20-slide batches were incubated with a primary monoclonal antibody to PrP^{res},F99/97.6.1(VMRD Inc., WA, USA) which was diluted 1:1500 in Dako® antibody diluent(Dako Canada Inc., ON, Canada) in an automated immunohistochemistry stainer (Benchmark™, Ventana Medical Systems Inc., AZ, USA) at 37 °C for 32 min. After washing slides with Ventana reaction buffer (Ventana Medical Systems Inc., AZ, USA), Horseradish Peroxidase (HRPO) conjugated secondary antibody (Ventana ultraView diamino benzidine

detection kit, Ventana Medical Systems Inc., AZ, USA) was applied on slides before 8 min incubation. Then, slides were washed with Ventana reaction buffer to remove excess antibody solution. Diamino benzidine (DAB) chromogen was smeared on slides and then those were incubated for 8 min. Again slides were washed using Ventana reaction buffer to clean additional chromogen on slides. Then Ventana Nexes Hematoxylin (Ventana Medical Systems Inc., AZ, USA) and Ventana Bluing Agent (Ventana Medical Systems Inc., AZ, USA) were used to counter-stain slides for 4 min each. Slides were then thoroughly washed in 5% dish washing soap (Dawn®, Proctor and Gamble, OH, USA) and rinsed in tap water. Then slides were immersed further in a series of graded alcohol and cleared in xylene before applying xylene-based embedding agent (Entellan®, Electron Microscopy Sciences, PA,USA). Finally tissue sections were examined under a light microscope for typical red staining in the lymphoid follicles of tonsils which was indicative of a positive case for PrP^{res}. A minimum of 6 follicles needed to be present before a sample was classified as negative (Gavier-Widen et al. 2005, Fernando 2010). If less number of follicles were present, those cases were finalized as inconclusive. Slides of positive cases as well as cases with high amounts of background staining were further processed with formic acid, proteinase 3 and hydrated autoclaving to confirm initial diagnosis.

3.3.5 Statistical analysis

All analyses except 95% confidence intervals for annual prevalence were conducted using SPSS statistical software (IBM SPSS statistics 19, IBM Corporation, NY, USA). Binary logistic regression was used to evaluate the covariate effects of sex and capture year for each infectious agent. Deer from capture year 2008 were not included in statistical analysis due to very low sample size (n=3) when comparisons were made among capture years with 2007 as the reference

year. *Trichostrongyloidea*, *Cryptosporidium* sp., flukes and *Skrjabenema* sp., which had a prevalence ≤ 1%, were not analyzed. Significance level was set at $\alpha = 0.05$.

Confidence interval for annual prevalence was calculated using STATA statistical software (STATA 12, StataCorp LP, TX, USA). When annual prevalence was zero, 95% confidence interval was calculated following 'rule of three' (Hanley and Lippman-Hand 1983).

Table 3-1: Summary of tests performed for each sample collected from 8-month old juvenile mule deer from 2007 to 2011 for selected infectious agents.

Sample	Test	Infectious agent/s	Total samples tested
Feces	Fecal flotation	Parasitic eggs	93
	Baermann technique	Parasitic larvae	93
	Fluorescent antibody test	*Giardia* cysts	89
		Cryptosporidium oocysts	89
	Fluke test	Fluke eggs	78
Tonsillar biopsy	Immunohistochemistry	Infectious prions for CWD	95
Serum	Serum neutralization test	1. Antibodies for bovine viral diarrhea virus	93
		2. Antibodies for parainfluenza 3 virus	93
	Competitive Enzyme Linked Immunosorbant Assay	1. Antibodies for bovine herpes virus-1	92
		2. Antibodies for *Neospora caninum*	91
Buffy coat	Conventional polymerase chain reaction	Mule deer lymphotropic herpes virus	85

Table 3-2: Summary of tests performed for each sample collected from neonatal mule deer from 2009 to 2011 for selected infectious agents.

Sample	Test	Infectious agent/s	Total samples tested
Serum	Competitive Enzyme Linked Immunosorbant Assay(cELISA)	Antibodies for *Neospora caninum*	106
Buffy coat	Real time polymerase chain reaction	Bovine viral diarrhea virus(BVDV)	85

3.4 Results

3.4.1 Juveniles

3.4.1.1 Parasitology

We analyzed feces from a total of 93 juveniles from 2007 to 2011. The prevalence of infection with *Orthostrongylus* larvae was highest at 86% (80/93) and this was consistent among years (Table 3-3, Table 3-5). Infection with cestodes was less common with 29% (27/93) of fecal samples containing *Moniezia* sp. and *Thysanasoma* sp.. The prevalence of *Eimeria* and *Trichuris* eggs were 10% (9/93) and 6% (6/93), respectively, all other parasites had a prevalence of less than 5%. Fluke eggs were not found in any of the samples (n =78) tested from 2007 to 2011. Mean intensity of infection per individual and range of infection is shown in table 3-4.

Moreover, *Nematodirus* [4.2% (2/48)], *Skrjabinema* [2.1% (1/48)], and *Crypstosporidium* [2.2% (1/45)] were detected only in male deer (Table 3.3) while *Trichostrongyloidea* was found in a single female in 2007 (Table 3.3, Table 3.5). The prevalence of parasitism was not significantly different between sexes ($P > 0.05$) (Table 3-6). However, for *Trichuris* ($P = 0.077$, OR= 0.170), *Moniezia* ($P = 0.669$, OR= 0.822), and *Giardia* ($P = 0.544$, OR= 0.477), the odds of females being infected were less than that of males although these differences were not statistically significant.

Only *Orthostrongylus* larvae, *Moniezia* and *Thysanasoma* eggs were found in juvenile fecal samples in all the capture years (Table 3-5). *Trichuris* eggs and *Eimeria* oocysts were prevalent in 4 out of 5 capture years. In detailed comparisons between years from logistic regression for *Orthostrongylus* prevalence, a higher possibility of infection occurred in 2011 deer compared to 2007 deer (P=0.004, OR=23) and in 2010 (P =0.000, OR=1.82) compared to 2007 while prevalence was similar between 2009 (P = 0.432) and 2007 (Table 3-7, Table 3-8).

Table 3-3: Overall prevalence and prevalence between sexes for fecal parasites collected from 8-month-old mule deer from 2007 to 2011, inclusive.

Parasitic agent		Overall prevalence	Prevalence between sexes	
			Female	Male
Nematode	*Orthostrongylus*	86%(80/93)	86.7%(39/45)	85.4%(41/48)
	Nematodirus	2%(2/93)	0%(0/45)	4.2%(2/48)
	Trichuris	6%(6/93)	11.1%(5/45)	2.1%(1/48)
	Trichostrongyloidea	1%(1/93)	2.2%(1/45)	0%(0/48)
	Skrjabanema	1%(1/93)	0%(0/45)	2.1%(1/48)
Cestode	*Moniezia*	29%(27/93)	31.1%(14/45)	27.1%(13/48)
	Thysanasoma	29%(27/93)	28.9%(13/45)	29.2%(14/48)
Protozoa	*Eimeria*	10%(9/93)	8.9%(4/45)	10.4%(5/48)
	Giardia	3%(3/89)	4.5%(2/44)	2.2%(1/45)
	Cryptosporidium	1%(1/89)	0%(0/44)	2.2%(1/45)
Trematode	Flukes	0%(0/78)	0%(0/37)	0%(0/41)

Table 3-4: Range and mean intensity of internal parasites (eggs, oocysts) in juvenile mule deer from 2007 to 2011, inclusive. Units – eggs/oocysts/larvae per 1g of feces.

Parasitic agent		Range	Mean intensity
Nematodes	*Orthostrongylus*	0 - 686	60.38
	Nematodirus	0 - 2	0.03
	Trichuris	0 - 3	0.10
	Trichostrongyloidea	0 - 0.4	0
	Skrjabanema	0 - 0.4	0
Cestodes	*Moniezia*	0 - 120	16.04
	Thysanasoma	0 - 19	1.42
Protozoans	*Eimeria*	0 - 27	0.44

Table 3-5: Prevalence of fecal parasites in juvenile mule deer from 2007 to 2011. 95% Confidence interval (95% CI) was stated with prevalence. CI was not calculated for 2008 (*n*=3).

Parasitic agent	Prevalence (95% CI)				
	2007	2008	2009	2010	2011
Orthostrongylus	55%(11/20) (31-77%)	100%(3/3)	70%(7/10) (35-93%)	100%(30/30)	96.7%(29/30) (82-100%)
Nematodirus	0%(0/20) (0-15%)	0%(0/3)	0%(0/10) (0-30%)	6.7%(2/30) (0-22%)	0%(0/30) (0-10%)
Trichuris	5%(1/20) (0-25%)	0%(0/3)	10%(1/10) (0-44%)	3.3%(1/30) (0-17%)	10%(3/30) (2-26%)
Trichostrongyloidea	5%(1/20) (0-25%)	0%(0/3)	0%(0/10) (0-30%)	0%(0/30) (0-10%)	0%(0/30) (0-10%)
Skrjabanema	5%(1/20) (0-25%)	0%(0/3)	0%(0/10) (0-30%)	0%(0/30) (0-10%)	0%(0/30) (0-10%)
Moniezia	35%(7/20) (12-57%)	66.7%(2/3)	30%(3/10) (0-64%)	36.7%(11/30) (18-55%)	13.3%(4/30) (0-26%)
Thysanasoma	25%(5/20) (4-46%)	66.7%(2/3)	40%(4/10) (3-77%)	30%(9/30) (13-47%)	23.3%(7/30) (7-39%)
Eimeria	20%(4/20) (0-39%)	33.3%(1/3)	0%(0/10) (0-30%)	6.7%(2/30) (1-22%)	6.7%(2/30) (1-22%)
Giardia	0%(0/19) (0-16%)	0%(0/3)	20%(2/10) (0-50%)	3.3%(1/30) (0-17%)	0%(0/30) (0-10%)
Cryptosporidium	0%(0/19) (0-15%)	0%(0/3)	0%(0/10) (0-30%)	0%(0/30) (0-10%)	3.3%(1/30) (0-17%)
Flukes	0%(0/5) (0-60%)	0%(0/3)	0%(0/10) (0-30%)	0%(0/30) (0-10%)	0%(0/30) (0-10%)

Table 3-6: Summary of statistical analysis for parasitic agents between sexes in juvenile mule deer from 2007 to 2011 pooled data (analysis was not performed for parasitic agents with a prevalence ≤1%), OR= odds ratio, CI= 95 % confidence interval for OR.

Parasitic agent	*P* value	OR (CI)
Orthostrongylus	0.862	0.901 (0.278-2.919)
Nematodirus	0.166	1.043 (0.984-1.107)
Trichuris	0.077	0.170 (0.019-1.518)
Moniezia	0.669	0.822 (0.336-2.016)
Thysanasoma	0.976	1.014 (0.414-2.483)
Eimeria	0.803	1.192 (0.299-4.750)
Giardia	0.544	0.477 (0.042-5.462)

Table 3-7: Summary of statistical analysis for parasitic agents between capture years in juvenile mule deer from 2007 to 2011 pooled data. Analysis was not performed for parasitic agents with a prevalence ≤1% and capture year 2008(n=3).

Parasitic agent	*P* value
Orthostrongylus	0.043
Nematodirus	1.000
Trichuris	0.744
Moniezia	0.440
Thysanasoma	0.213
Eimeria	0.761
Giardia	0.500

Table 3-8: Summary of pair-wise comparison among capture years for prevalence for *Orthostrongylus* larvae in feces of 8-month-old mule deer from 2007 to 2011.

Parasitic agent	2009 compared to 2007	2010 compared to 2007	2011 compared to 2007
Orthostrongylus	*P* = 0.432	*P* = 0.000 OR = 1.82 CI = 1.22-2.7	*P* = 0.004 OR = 23.7 CI = 2.6-209.7

3.4.1.2 Viral agents

Overall percentage of juvenile deer with positive BHV-1 titers was 3% (3/92). BHV-1 titers were only detected in 2010 (10% (3/30). Males had a prevalence of 6.3% (3/42) and none of the females had antibodies to BHV-1 (0/42) in 2010 (Table 3-9, Table 3-10). When comparisons were made between sexes, controlling for year: female [0% (0/44)] and male [6.3% (3/42)] (P=0.998) (Table 3-11) or capture years controlling for sex: 2007(0/19, 0%), 2009(0/9, 0%), 2010(3/30, 10%) and 2011(0/31, 0%) (P=1.000) (Table 3-10, Table 3-11), there was no significant difference in prevalence of BHV-1 titers.

Overall prevalence for BVDV antibodies was 20% (20/93) for all capture years (Table 3-9). No difference was observed between females [23% (10/44)] and males [18% (9/49)] for BVDV antibody prevalence (P= 0.438) when controlled for year (Table 3-11). Prevalence for BVDV was 5% (1/19) in 2007; 0% (0/3) in 2008, 33% (3/9) in 2009, 40% (12/30) in 2010, 10% (3/31) in 2011 (Table 3-10). However; a significant difference was observed between capture years on exposure to BVDV from binary logistic regression when controlled for sex (P= 0.008, df= 3) (Table 3-11). In 2010, BVDV antibody prevalence was significantly higher compared to 2007(P= 0.016) with a higher risk of being infected in 2010 than 2007 (OR=13.7) (Table 3-12). BVDV antibody prevalence was similar between 2009 and 2007(P=0.078) as well as 2011 and 2007(P=0.602) when pair-wise comparisons were made between years. In 2010, seropositive juveniles showed high titers (Figure 3-1).

Overall prevalence of deer exposure to PI3 was 22% (20/93) from 2007 to 2011(Table 3-9). Male juveniles [24.5% (12/49)] showed a higher prevalence for exposure to PI3 than females [18.2% (8/44)] though it was not a significant difference statistically (P= 0.461) (Table 3-11). However, PI3 antibody prevalence had a significant difference among capture years (P= 0.000) (Table 3-11). Total number of individuals which had different titers for PI3 in each capture year

are shown in Figure 3-2. All the capture years 2009 (*P*= 0.005, OR=0.33), 2010 (*P*= 0.00, OR=0.009), 2011(*P*= 0.00, OR=0.211) showed significantly lower infection probabilities for PI3 compared to 2007 as detected by serology (Table 3-12).

Table 3-9: Prevalence for exposure to selected infectious agents among sexes in juvenile mule deer detected by serology from 2007 to 2011 pooled data.

Exposure to infectious agent	Overall prevalence	Prevalence between sexes	
		Female	Male
BHV-1	3%(3/92)	0%(0/44)	6%(3/42)
BVDV	20%(19/93)	23%(10/44)	18%(9/49)
PI3	22%(20/93)	18%(8/44)	24%(12/49)
Neospora caninum	3%(3/91)	2%(1/43)	4%(2/48)

Table 3-10: Annual prevalence for exposure to selected infectious agents in juvenile mule deer detected using serology from 2007 to 2011. 95% Confidence interval (95% CI) was stated with prevalence. CI was not calculated for 2008 (*n*=3).

Antibodies to infectious agent	Prevalence (95% CI)				
	2007	2008	2009	2010	2011
BHV-1	0%(0/19) (0-15%)	0%(0/3)	0%(0/9) (0-33%)	10%(3/30) (0-21%)	0%(0/31) (0-10%)
BVDV	5.3%(1/19) (0-26%)	0%(0/3)	33.3%(3/9) (7-70%)	40%(12/30) (23-60%)	9.4%(3/32) (2-25%)
PI3	78.9%(15/19) (54-93%)	100%(3/3)	11.1%(1/9) (0-48%)	3.3%(1/30) (0-17%)	0%(0/32) (0-10%)
Neospora caninum	11.8%(2/17) (1-36%)	0%(0/3)	0%(0/9) (0-33%)	0%(0/30) (0-10%)	3.1%(1/32) (0-16%)

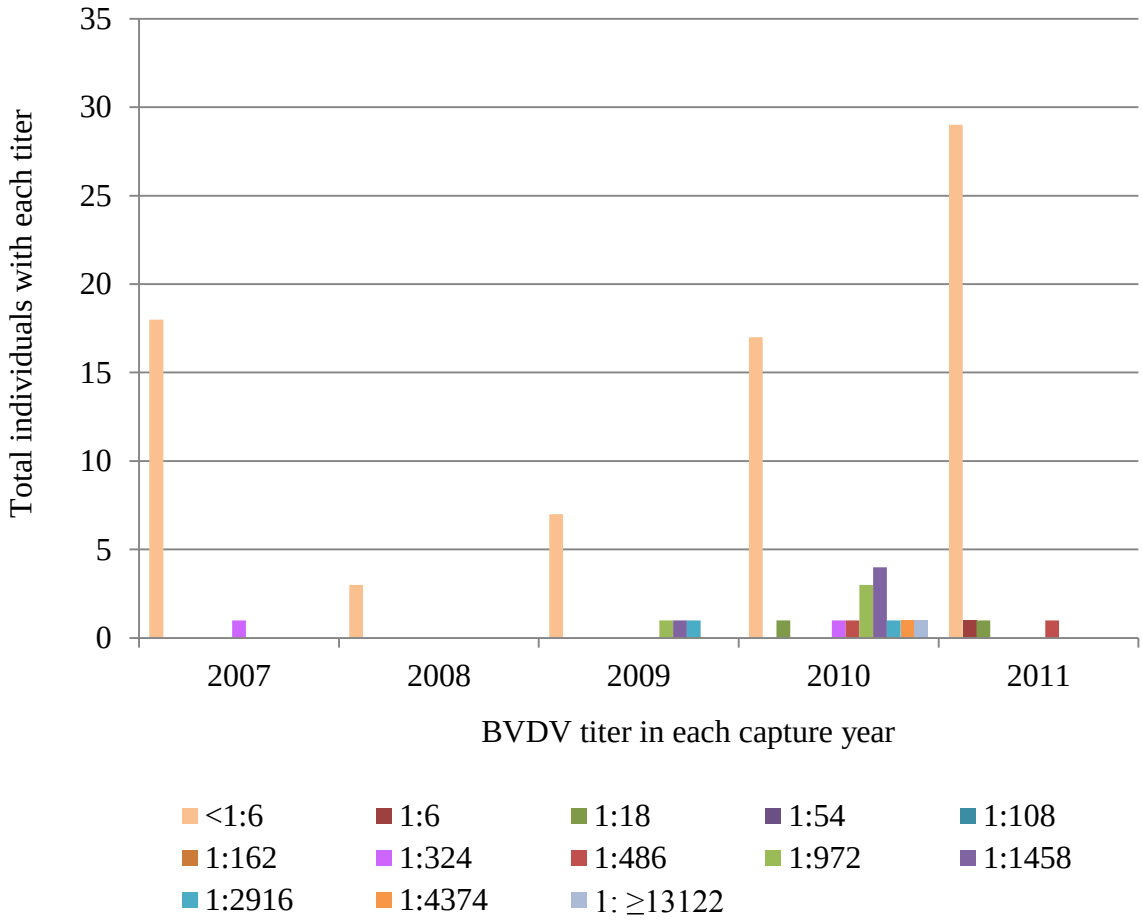

Figure 3-1: Total mule deer juveniles with different titers for bovine viral diarrhea virus (BVDV) as detected by serum neutralization from 2007 to 2011. Titer of <1:6 was considered negative, titer of ≥1:6 was considered positive.

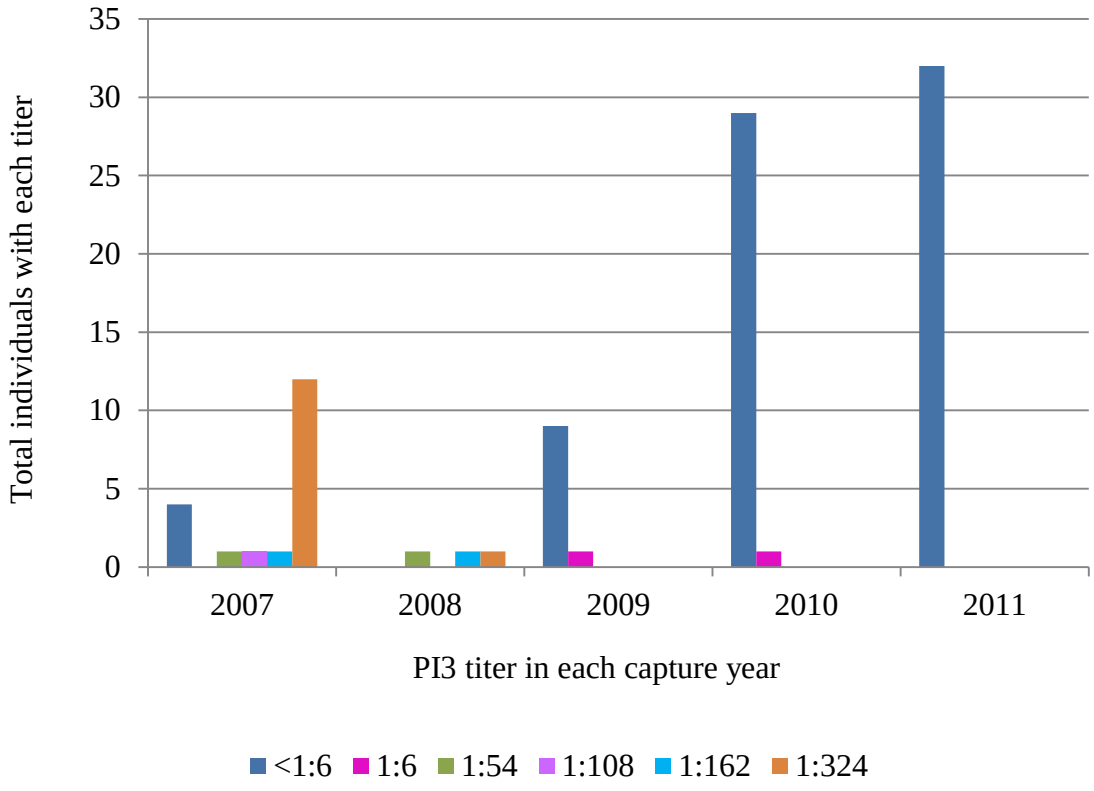

Figure 3-2: Total mule deer juveniles with different titers for parainfluenza3 virus (PI3) as detected by serum neutralization from 2007 to 2011. Titer of <1:6 = seronegative, titer of ≥1:6 = seropositive.

Table 3-11: Summary of binary logistic regression analysis for prevalence of antibodies to selected infectious agents between sexes (when controlled for year) and among capture years (when controlled for sexes) in mule deer juveniles from 2007 to 2011, df = degrees of freedom.

Antibodies for infectious agent	*P* value (between sexes, df=1)	*P* value (between years, df=3)
BHV-1	0.998	1.000
BVDV	0.438	0.008
PI3	0.461	0.000
Neospora caninum	0.628	0.100

Table 3-12: Summary of pair-wise comparison between capture years on prevalence of antibodies for bovine viral diarrhea virus (BVDV) and parainfluenza 3 (PI3) virus obtained by serology for juvenile mule deer from 2007 to 2009.

Antibodies for Infectious agent	2009 compared to 2007	2010 compared to 2007	2011 compared to 2007
BVDV	*P* = 0.078	*P* = 0.016 OR= 13.7 CI= 1.62-116.9	*P* = 0.602
PI3	*P* = 0.005 OR= 0.33 CI= 0.003-0.351	*P* = 0.00 OR= 0.009 CI= 0.001-0.090	*P* = 0.000 OR= 0.211 CI= 0.088-0.503

3.4.1.3 *Neospora caninum*

The total percentage of mule deer juveniles exposed to *Neospora caninum* from 2007 to 2011was 3% (3/91) (Table 3-9). The prevalence in female juveniles was 2.3% (1/43) while in males it was 4.2% (2/48) (Table 3-9). The prevalence of *Neospora* titers was not different between sexes (P= 0.628) (Table 3-11). Out of the 5 capture years, *Neospora* was only detected in 2007 [11.8% (2/17)] and 2011 [3.1% (1/32)] and there was no difference in prevalence between these years (P= 0.100) (Table 3-11).

3.4.1.4 Chronic Wasting Disease

We tested a total of 95 juvenile mule deer for CWD from 2007 to 2011, inclusive (Table 3-13). In 2007, 2009 and 2010, the number of juveniles with an inconclusive CWD result was 2, 2, and 3, respectively. All samples tested from 2007 to 2009 (except cases with inconclusive results) were negative for chronic wasting disease. In 2010, 2 (1 female and 1 male) out of 30 samples were positive. Both of these deer died of CWD: the female at approximately 22 months of age and the male at approximately 28 months of age. The dams of these deer were uncollared and therefore their CWD status was unknown. In 2011, the single positive tonsillar sample was obtained from a juvenile female born to a CWD positive, collared, adult female. This juvenile died within 2 weeks of capture. Most of the carcass had been scavenged including the brain but the spinal cord was negative for CWD with IHC. Thus, CWD positive juvenile percentage (excluding inconclusive cases) was 7% (2/27) and 3% (1/32) in 2010 and 2011 respectively making the overall percentage for all the capture years 3% (3/88) among 8-month olds. In 2010, 25/27 (93%) 8-month old deer were negative for CWD while 31/32 (97%) were negative in 2011.

Table 3-13: Immunohistochemistry results for chronic wasting disease (CWD) performed on tonsillar biopsies obtained from 8-month-old mule deer captured from 2007 to 2011. CWD positive % is calculated excluding inconclusive cases.

Capture year	Total samples tested	CWD negative samples	Inconclusive samples	CWD positive samples	CWD positive %
2007	20	18	2	0	0
2008	3	3	0	0	0
2009	10	8	2	0	0
2010	30	25	3	2	7.4
2011	32	31	0	1	3
Total	95	85	7	3	3.4

3.4.1.5 Mule Deer Lymphotropic Herpes Virus (Mule deer – LHV)

We tested 85 buffy coat samples from juvenile deer for mule deer-LHV from 2007 to 2011 and 15% (13/85) were positive and 85% (72/85) were negative (Figure 3-3). From females, 15.4% (6/39) were positive for mule deer-LHV and 84.6% (33/39) were negative. Seven out of 46 males (15.2%) were positive for LHV while 84.8% (39/46) were negative. The prevalence of LHV infection did not differ between the sexes ($\chi_1^2 = 0.00$, $P = 0.983$). There was a significant difference in mule deer-LHV prevalence among years ($P = 0.040$). On further analysis, this difference occurred between 2010 compared to 2007 ($P= 0.010$) and 2011 compared to 2007 ($P= 0.015$) but not between 2009 and 2007 ($P= 0.999$) from binary logistic regression. Compared to 2007, both 2010 and 2011 years, 8-month olds had a lower risk of obtaining mule deer-LHV infection according to odds ratios which were 0.115 and 0.148 respectively.

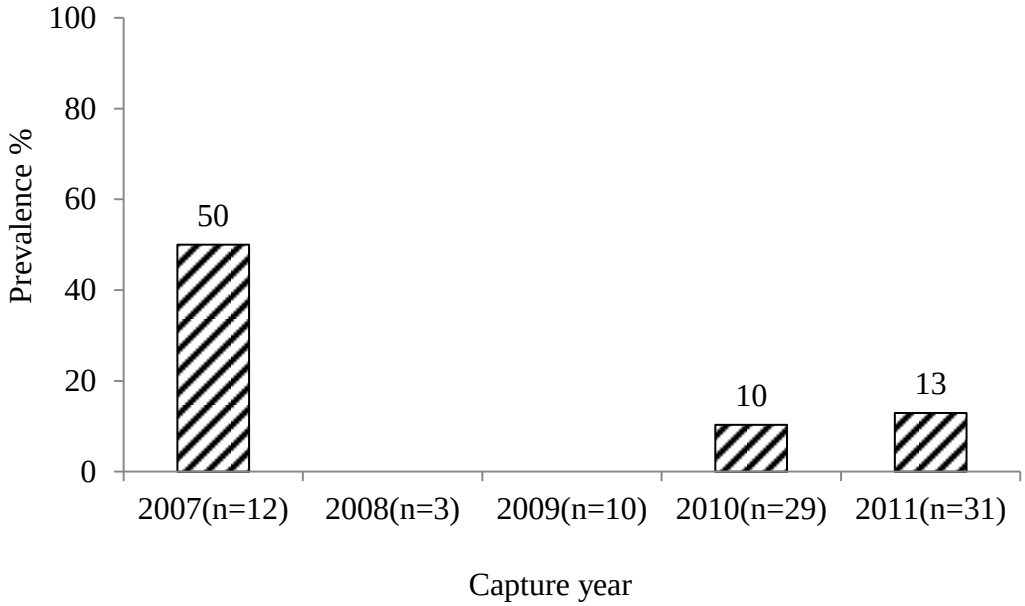

Figure 3-3: Prevalence of mule deer lymphotropic herpes virus (mule deer-LHV) for juvenile mule deer captured from 2007 to 2011 detected by conventional polymerase chain reaction.

3.4.2 Neonates

3.4.2.1 Bovine viral diarrhoea virus (BVDV)

Out of 85 buffy coat samples tested from neonates from 2009 to 2011, we found one female fawn in 2009 positive for BVDV using real time PCR. This fawn was believed to be drowned at 5 months of age and no remains were available. We tested tonsillar tissue of dam of this neonate for persistent infection and she was negative from immunohistochemistry.

3.4.2.2 *Neospora caninum*

Antibody titers to *Neospora caninum* were present in 12% (13/106) of neonates from 2009 to 2011. The prevalence of titers in female neonates [16.7% (9/54)] was twice that of their male counterparts [7.8% (4/51)] (OR=2.35) but this difference was not significant ($P = 0.179$). We further found that neonates had immunoglobulin to *Neospora* in all capture years from 2009 to 2011: 14.7% (5/34) in 2009, 8.1% (3/37) in 2010 and 14.3% (5/35) in 2011. However; no difference occurred among years for neonate prevalence for *Neospora* titers ($P = 0.640$).

We further tested serum (collected in winter, approximately 3 months prior to neonate sample collection) of dams of all neonates to determine any effects on reproduction by *Neospora* infection. From 2009, 2010 and 2011 winter sampling, 22% (2/9), 13%(5/39) and 9%(4/44) pregnant does were found to be positive for *Neospora* antibodies and all seropositive females (except 1 doe each in 2010 and 2011 which had moved out of study area before fawn capture for which no data was available on fawn production) gave birth to fawns. Furthermore, all neonates (n=12) which were born to seropositive females carried immunoglobulin for *Neospora* indicating effective passive transfer of immunity through colostrum. For one seropositive neonate, corresponding dam sample was not available.

3.5 Discussion

This study reveals the presence of several gastro-intestinal parasites: *Orthosrongylus* sp., *Nematodirus* sp., *Trichuris* sp., Trichostrongyloidea sp., *Skrjabanema* sp., *Moniezia* sp., *Thysanasoma* sp., *Eimeria* sp., *Giardia* sp., and *Crypstosporidium* sp. in juvenile mule deer in southern Saskatchewan. Mule deer juveniles were infected with *Orthostrongylus* sp., *Moniezia* sp. and *Thysanasoma* sp. in all capture years. However, clinical signs were not associated with any of these infections. *Orthostrongylus* sp. had the highest prevalence compared to other parasites over the entire 5 years of the study. Furthermore, we found moderate to low prevalence for *Moniezia* sp. and *Thysanasoma* sp. eggs during fecal examination. Fernando (2010) reported higher parasitic prevalence in juveniles compared to adults in mule deer in a larger study area in southern Saskatchewan which included Antelope Creek.

Although we did not determine the species of *Orthostrongylus*, they are most likely *O. macrotis*, a species reported elsewhere in Rocky Mountain mule deer (Dougherty and Goble 1946, Pybus 1990, Robb and Samuel 1990). We used morphology to identify parasitic larvae extracted from fecal matter. Larvae of *Orthostrongylus macrotis* have straight and symmetrical posterior ends whereas *Parelaphostrongylus odocoilei* possess a dorsal spine (Ballantyne and Samuel 1984, Robb and Samuel 1990). The prevalence of *Orthostrongylus* sp. was significantly higher during the last two capture years compared to 2007 which could be a consequence of higher precipitation in 2010 and 2011. Higher precipitation may have facilitated increased production and survival of gastropod intermediate hosts of *Orthostrongylus* sp. According to Fernando (2010), lungworms were present in only 31% of adult mule deer feces in late winter in southern Saskatchewan. Given the high prevalence in juveniles this would suggest adults clear the infection, or only shed larvae intermittently during the winter.

Fernando (2010) did not detect *Cryptosporidium* sp. or *Giardia* sp. in fecal samples using FAT from adult or juvenile deer in the area of the South Saskatchewan River during 2006 to 2008. However, we detected *Cryptosporidium* sp. oocysts using FAT in one juvenile male as well as *Giardia* sp. from 3 juvenile deer. Rickard et al. (1999) found in Virginia and Mississippi that white-tailed deer juveniles less than 6 months of age shed *Cryptosporidium* oocysts and *Giardia* cysts and infection probability was inversely proportional to deer age. *Cryptosporidium* sp. and *Giardia* sp. are considered to be important food or water borne pathogens which may lead to serious disease conditions in humans as well as in animals (Guerrant 1997, Thompson et al. 2008).

In Antelope Creek, we detected only very low prevalence of *Eimeria*, *Nematodirus*, *Trichuris,* and *Skrjabenema* infections. Since we collected fecal samples in the late winter these prevalence estimates are likely low as intensity of infective oocyst, egg or larvae shedding varies seasonally and is typically lowest in the winter (Robb and Samuel 1990, Hoberg et al. 2001, Jenkins et al. 2006). Consistent with Fernando (2010) and Wobeser et al. (1985), we did not detect liver fluke or other trematode eggs in any fecal sample from juveniles.

In 2010 and 2011, we detected 2 and 1 subclinically CWD infected mule deer juveniles, respectively. Due to the long incubation period of CWD infections, it is not common to detect juveniles infected with CWD and therefore, most targeted surveillance programs, including that in Saskatchewan, concentrate more on CWD infection in adult deer. However, in Wisconsin, six white-tailed fawns had shown subclinical infection for CWD with a prevalence of 0.5% as reported by Grear et al. (2006). PrP^{CWD} was detected as early as 42 days after orally feeding brain homogenate of naturally infected deer to mule deer fawns (Sigurdson et al. 1999). Vertical passage of infectious prions from dam to offspring is rare (Miller and Williams 2003) and little is

known about this route of infectious prion transfer in cervids. Horizontal transmission remains the most common mode of transmission among infected and susceptible individuals for CWD and maternal transmission is less likely to contribute to disease spread (Miller and Williams 2003). The minimum incubation period for CWD is considered to be at least 16 months and thus clinical disease is rare in young deer (Williams 2005). CWD prevalence in the Antelope Creek study area has increased to 33% according to 2011 winter surveillance (CCWHC 2011). Evidence of subclinical CWD infection in juveniles may indicate noteworthy increase of environment contamination by abnormally folded PrP^{CWD} which is expected with a rising prevalence in adults in the study area. Although the single juvenile female we encountered to be CWD positive in 2011 was born to a CWD positive female, we cannot be certain about the route by which she contracted infection as we do not have any information on her infection status at birth. It is highly likely that growing up in the surroundings of an infected dam which continually created a highly contaminated environment, undoubtedly magnified the odds of lateral acquisition of abnormally transformed infectious prion particles.

We did not observe any variation in exposure to BHV-1, BVDV, or PI3 between sexes. However, exposure varied among years for BVDV and PI3 over the 5 years of the study. BVDV antibodies were low in 2007, peaked among juvenile deer in 2010 (40%) and dropped again by the 2011 winter. Myers (2001) observed similar variation in prevalence, from 4% to 24%, among wintering juvenile mule deer in two study years in western Colorado. Among neonatal mule deer, we detected a single fawn in 2009, which was potentially infected with BVDV although we could not confirm this as it was not possible to obtain serial samples from this fawn. It is probable that there was an acute infection outbreak among cattle and/or deer in the area which led to subclinical, acute or persistent infections in deer. Recently Passler et al. (2007), Duncan et

al. (2008a) and Duncan et al. (2008b) documented persistent BVDV infection among captive and wild deer in North America. If the infection wave created any persistently infected deer, probably continuous environmental contamination and reinfection of susceptible individuals could have lasted for several years before the infection wave died down. For PI3 antibodies, prevalence which was more than 70% in 2007 and 2008, dropped drastically to 11% in 2009 and continued to die down. However, it is important to mention that all 3 juvenile deer positive for PI3 antibodies in 2008 could possibly have over represented true exposure rates to PI3 that year. Sadi et al. (1991) documented consistency in seroprevalence for PI3 in white-tailed deer in all age categories from 1985-1987. Ingebrigtsen et al. (1986) reported 24% and 11% seroprevalence to PI3 in Minnesota white-tailed deer among adults and fawns respectively. Ingebrigtsen et al.(1986) and Fernando (2010) reported seroprevalence to PI3 was significantly higher prevalence in adults compared to juveniles. Lower antibody prevalence reported commonly for fawns or juveniles could be due to a shorter window of exposure and limited movements during early stages of life compared to mature animals. Without proper scientific knowledge on how these agents are represented in the cattle populations in the study area which often share the grazing grounds with wildlife, we may not be able to fully understand the yearly trends in exposures for different viruses in Antelope Creek.

Prevalence for BHV-1 and *Neospora caninum* among mule deer juveniles was zero or very low throughout the years. In Quebec, Sadi et al. (1991) observed a decreasing trend in seroprevalence in white-tailed deer adults and fawns after a BHV-1 epizootic during the 3 years of their study. Cantu et al. (2008) detected antibody prevalence to BHV-1 as high as 41% in northeastern Mexico in white tailed deer. Ingebrigtsen et al.(1986), Uhart et al. (2003) and Yokoi et al.(2009) reported less than 10% prevalence of antibodies to BHV-1 in different deer species.

The annual proportion of neonates, juveniles and adult females which had serum antibodies to *Neospora caninum* was ≤ 15%. All females which were exposed to *Neospora,* gave birth to live fawns which implied that *Neospora* infection had little or no effect on mule deer reproduction. In a parallel study on female reproduction and fawn survival (Chapter 1), we observed that all females were pregnant with an average fetal count of 2 fetuses per doe, however; birth rate was 1.3 fawns per doe for 3 years. Although we could not see any effect of infectious causes on female production, this difference in prenatal and postnatal fawn production could have been a combined result of late pregnancy fetal losses (Runge and Wobeser 1975, Pojar and Bowden 2004) possible errors in fetal counting by transabdominal ultrasound scanning (Smith and Lindzey 1982, Stephenson et al. 1995) as well as high neonatal mortality within 24 hours of birth which is often undetected in wild (Pojar and Bowden 2004).

Mule deer lymphotropic herpes virus (mule deer-LHV) belongs to the genus *Rhadinovirus* in the subfamily *Gammaherpesvirinae* and are further categorized as type 2 ruminant rhadinovirus (type 2 RuRV). Existence of mule deer-LHV was first reported recently by Li et al. (2005) based on molecular characterization of DNA polymerase gene sequences. Most recently Fernando (2010) reported that 42% of mule deer in southern Saskatchewan were infected with mule deer-LHV. Furthermore, the same author identified a yearly reduction in prevalence from 48% in 2007 to 28% in 2008 which was statistically significant although no prevalence difference was noted between adult and juvenile deer. In this study, we noticed a similar varying trend but with a lower prevalence for mule deer-LHV among juvenile deer between years, after 2007. Members of *Gammaherpesvirinae* subfamily tend to establish latency immediately after initial infection in various sites including lymph nodes, mononuclear blood cells, ganglia, and bone marrow (Ackermann 2006). Fernando (2010) demonstrated presence of mule deer-LHV in

retropharyngeal lymph nodes as possible sites for dormancy in mule deer and white-tailed deer in addition to peripheral blood lymphocytes. Varying degrees of dormancy in lymphoid tissues may be responsible for variations in prevalence over the years in addition to other unknown host and environmental factors which may determine degree of latency. Clinical significance of type 2 RuRVs which include mule deer-LHV, elk-LHV, fallow deer-LHV, black-tailed deer-LHV, oryx-LHV, domestic sheep-LHV, bighorn sheep-LHV, bison-LHV, domestic goat-LHV is not clearly understood. However, Squires et al.(2012) detected type 2 RuRV together with cervid herpes virus 1 in conjunctiva of a farmed red deer (*Cervus elaphus*) with ocular lesions as well as ones without eye disease. Significance of type 2 RuRV in this case remained undetermined. Depressed immune system function in a host may prompt termination of latency and trigger viral replication which may result in clinical disease (Ackermann 2006).

We estimated rates of exposure to infectious prions causing CWD and other selected infectious agents of juvenile mule deer in CWD-endemic southern Saskatchewan. Together with increasing CWD prevalence in adult deer, detection of subclinical CWD infection in 8- month-old juvenile deer in spite of prolong incubation period suggest, high infection pressure in the study area. We selected other infectious agents which had similar routes of transmission to CWD: gastrointestinal parasites which spread through contact with contaminated environmental and viral agents which transmit through direct contact between infected. High prevalence of *Orthostrongylus* sp. lung worm larvae and a moderate prevalence of *Moniezia* sp. and *Thysanasoma* sp. cestode eggs in the feces of juvenile deer found during this study indicate moderate to high fecal contamination of the environment. Further studies on degree of exposure to these infectious agents in livestock which share resources with sympatric deer in the study area will be helpful in fully understanding annual variations in exposure to *Orthostrongylus* sp.,

BVDV, and PI3 found in this study. Because these infectious agents have shorter and less variable incubation periods compared to CWD, those will respond rapidly to management actions which target direct horizontal transmission and/or environmental exposure. Thus, baseline information on age and sex specific infection rates over multiple years reported in this study and Fernando (2010) will help wildlife managers to evaluate efficiency of such management plans within a short post-implementation period.

CHAPTER 4

GENERAL DISCUSSION

This study was designed to evaluate two different aspects of early life history in mule deer in a CWD endemic area in southern Saskatchewan. The primary objectives were 1) to estimate female reproductive indices and fawn survival, and 2) to determine the extent of exposure to infectious agents within the first 8 months of life. Reproductive and survival rates obtained from this study and estimates of baseline life history traits obtained from other parallel studies in the area (Silbernagel 2010, Skelton 2010), will be utilized in developing a model to predict the long term effects of CWD on this mule deer population.

We found that even though female pregnancy rate and fetal rate were high, fawn survival and recruitment were low in mule deer in Antelope Creek. Only about 1/3 of fawns survived to 8 months of age and most died of predation. Previous reports of mule deer fawn survival ranged from 16% to 52% (Bleich et al. 2006, Johnstone-Yellin et al. 2009) We found that fawn survival was lowest within 30 days of birth. Having a longer body length at birth was positively related to survival within a week of birth, while being born as a part of a larger litter was negatively related to survival at an early age. We demonstrated that radio-collar weight had a negative effect on neonate survival during the first week after birth and collars should be less than 2.5% of a fawn's body weight in order to minimize these effects. None of the other parameters related to handling, including handling time or age at capture, had any effect on fawn survival. It is crucial to assess and minimize the effects of handling and marking in survival studies.

Fawns of mothers that were positive for subclinical CWD had a lower survival probability than those having mothers that were CWD negative, 0.286 versus 0.391, respectively, but this was not statistically significant. Only a few recent studies have looked at effects of CWD on deer reproduction and recruitment (Dulberger et al. 2010, Blanchong et al. 2012). Blanchong et al.

(2012) reported that subclinical CWD infection had no effect on parenting ability of both male and female adult white tailed deer using hunter harvest deer samples in south-central Wisconsin. In our study we found that all females were pregnant and most were carrying twin fetuses, irrespective of their subclinical CWD status. However, when annual fawn recruitment was calculated at 6 months of age, it was apparent that females with subclinical CWD infection had lower recruitment compared to uninfected females. In Colorado, fawn recruitment at 2-3 months of fawn age was 0.22 fawns/doe for CWD infected dams and 0.32 fawns/doe for uninfected dams indicating infected dams produce 1.5 times fewer fawns (Dulberger et al. 2010).

In Wisconsin, fawns of females with subclinical CWD infection were more likely to be shot by hunters than were fawns of uninfected dams (Blanchong et al. 2012). Same authors proposed two reasons for this observation: 1) CWD positive dams were at higher risk of being shot by hunters and then subsequently their fawns and 2) infected does were less attentive to their fawns and therefore were easy targets for hunters. Similarly, we suggest that although we do not see any direct substantial effects of subclinical CWD infection on fawn survival, other possible behavioral changes in the dam which negatively affect parental care may indirectly lead to low offspring recruitment.

We detected subclinical CWD infection in 3/88 (3%) young deer of 9 months of age. In naturally infected deer, the incubation period for CWD is estimated to be a minimum of 16 months with an average of 2 to 4 years (Williams 2005). The accumulation of prions in tonsils to levels detectable by immunohistochemistry takes several months post-exposure. The incubation period of prion diseases is also dependent on the infectious dose and genetics of the individual infected (Johnson et al. 2011, Robinson et al. 2012, Wolfe et al. 2012). Our ability to detect subclinical infection in a number of deer by 8 months of age suggests early contact with PrP^{CWD};

either in utero, by contact with infected individuals or by contact with contaminated environments. The high and increasing CWD prevalence in adult mule deer in this population indicates that there is a high probability of exposure to CWD in the study area. We also detected a high prevalence of *Orthostrongylus* sp. lung worm larvae and a moderate prevalence of *Moniezia* sp. and *Thysanasoma* sp. cestode eggs in the feces of juvenile deer throughout the duration of the study. These parasites are transmitted to deer via intermediate hosts found in the soil, water and on vegetation. High prevalence of these parasites indicates high levels of fecal contamination of the environment. *Moniezia* sp. and *Thysanasoma* sp. have mites as their intermediate hosts and it has been reported that hay mites can transmit scrapie (Wisniewski et al. 1996, Carp et al. 2000). The potential role of mites in CWD transmission is worth investigating.

Changes in prevalence of antibody titers to BVDV over time, suggests the occurrence of an epizootic of bovine virus diarrhea in wild and/or domestic ruminant populations during the 2009/2010 period. Similarly, high prevalence of titers to PI3 in 2007 and 2008 followed by decline over the next few years suggests an epizootic of this disease in 2007-2008. Exposure of juveniles to BHV-1, *Neospora caninum* and mule deer-LHV was uncommon during the first 8 months of life. In order to fully understand the patterns of exposure and transmission of these agents in wild deer, it would be necessary to simultaneously study these infectious agents in sympatric cattle populations. Exposure of female mule deer to *N. caninum* did not seem to have an effect on fawn production in Antelope Creek.

4.1 Study limitations

We determined female reproductive indices and estimated of fawn survival for mule deer in southern Saskatchewan. The higher average fetal count compared to birth rates during all study years could be due to natural fetal deaths in late gestation and/or neonatal deaths within the first few hours of birth (Pojar and Bowden 2004), or due to errors in fetal counts during late

pregnancy (Smith and Lindzey 1982, Stephenson et al. 1995). Due to difficulties in locating newborn fawns we may have underestimated fawn numbers; however; we tried to improve our counts of live fawns born per female by using data from a parallel study directly observing behavior of deer including females and fawns.

For fawns born to uncollared females, females without VIT implants or where the VIT failed, we used average new hoof growth to back calculate the fawn age at capture following techniques described by Robinette (1973). We then estimated birth weight from capture weight using age at capture. Thus, errors in hoof growth measurement undoubtedly created further errors in birth weight calculations. For fawns born to females with functional VITs, errors in birth weight calculations were minimal as we used the hours since expulsion of VITs to calculate the age at capture.

In Chapter 3 we reported the apparent prevalence of selected infectious agents in mule deer neonates and juveniles. These results are dependent on test performance characteristics such as sensitivity, specificity, positive and negative predictive values and true prevalence of a particular agent in the population which may significantly affect predictive values of a diagnostic test performance especially when true prevalence is low (Banoo et al. 2008). Serological tests used in this study were primarily designed and validated for domestic cattle, not for wild species. Thus, seropositivity represents antibodies to the target agent plus other similar agents. For example, antibodies to non-BHV-1 *alphaherpesviruses* such as cervid herpes virus 1 in deer and cervid herpes virus 2 in reindeer can cross-react with test antigens for BHV-1 in serological tests (Lyaku et al. 1992). However, the ELISA used for detection of antibodies for *Neospora caninum* was specific and does not cross-react with other protozoan species such as *Toxoplasma gondii* or *Sarcocystis* species (Baszler et al. 1996). Also, serological tests are unable to detect animals that

have been infected but have not yet seroconverted at the time of sample collection (Philippa et al. 2008). For all these reasons, serological test results should be interpreted more cautiously than tests that directly detect presence of an infectious agent.

However, tests that detect infectious agents or infective stages may have their own limitations. Polymerase chain reactions determine the presence of minute amounts of nucleic acids in samples but the mere presence of nucleic acids may not always indicate the presence of viable infectious agent (Givens et al. 2002).

False negative results may occur for several reasons; such as, dormancy after initial infection (Capitini et al. 1990), or seasonal fluctuations in the production of infective stages (Bowman 2009). For example, results from fecal samples collected during winter in this study may not be indicative of the true prevalence of parasitism in this population as fecal shedding of larvae and eggs tends to be lowest in the winter.

As with many field studies, low samples sizes made it difficult to demonstrate statistical differences between and among groups, in some cases. In 2008, we were only able to obtain samples from three juvenile deer and, because of the low sample size, the confidence around our prevalence estimates were very large, making comparisons to other years impossible.

The study was also limited by not knowing the population size and demographics for mule deer in Antelope Creek during the years of our study. From the 1984-2003 period, the mean winter mule deer population in South Saskatchewan River management unit, that includes Antelope Creek, was reported to be 9620 (Arsenault 2005). In order to extrapolate our findings to a population perspective, it is essential to estimate population in the study area.

4.2 Management Implications

One of the objectives of this study was to estimate survival of mule deer in the first year of life. This estimate will to be combined with other age-sex class survival estimates, derived from previous studies in the area, to develop a model to estimate the effects of CWD on this population. We determined that only 33 % of fawns born in spring survived to first winter. High predator pressure on young deer was the main cause of mortality of fawns. Low recruitment rates mean that it would take approximately 5 years for a doe to replace herself and this, in combination with increasing losses due to CWD, could potentially lead to population declines. Research is underway to develop population models to estimate the effects of CWD in this area. It is likely, in the absence of strategies to reduce CWD transmission, population declines will occur unless management action is taken to reduce coyote predation on fawns and to reduce hunting pressure on does.

This study estimated the rates of exposure of mule deer within the first 8 months of life to infectious agents which have routes of transmission similar to that of CWD, gastrointestinal parasites via environmental exposure, and viral agents through direct horizontal transmission. The moderate to high prevalence of exposure to infectious agents in this first year supports the hypothesis that CWD transmission is likely, if CWD is present in the population and/or in the environment. The prevalence estimates of 3% for CWD at 9 months of age are likely low due to the inability to detect infection in the early stages of a long incubation period. If this assumption is correct, many of the juveniles that disperse at 12 and 18 months of age will be carrying CWD to new locations. Since this is likely the case, dispersal should be an important consideration in developing CWD management strategies in order to reduce rates of geographic spread of this disease.

Together with prevalence estimates for these infectious agents in adults reported by Fernando (2010), information from this study on juveniles provides a baseline of age-specific infection rates in this wild deer population. These selected infectious agents have shorter and less variable incubation periods compared to CWD, but share similar transmission routes. Thus, if management actions are implemented to control direct horizontal transmission and/or environmental exposure, those agents should take a comparatively shorter time to respond and help wildlife managers to evaluate the success or failure of those management plans more rapidly. Data collected on changes in exposure to these infectious agents over multiple years are likely to predict how CWD will respond to such management attempts but within a shorter post-implementation period (Fernando 2010).

Domestic livestock and wildlife share numerous pathogens and these pathogens continue to evolve in response to various selection pressures which can lead to emergence of diseases, especially where livestock and wildlife interphase occurs (Siembieda et al. 2011). In our study area, physical contact among cattle, wild deer and wild canid populations occurs for resources, such as rangeland, watering and feeding sites. According to this study and the study of Fernando (2010), seroprevalence to BVDV, BHV-1, PI3 and *Neospora* was detected in wild deer in southern Saskatchewan. BVDV infects domestic species such as cattle, sheep and goats and many wildlife species, causing reproductive disease (Ridpath et al. 2008, Ridpath 2010). BHV-1 and PI3 cause respiratory disease in cattle. *Neospora* infection results in cattle abortions and wild canids act as definitive hosts in the parasitic life cycle (Dubey and Schares 2011). In shared environments, these infectious agents can readily transmit from cattle to sympatric wild animal populations and vice versa. Long-term studies of such agents in all relevant sympatric species

help in understanding the ecology of these infectious agents and ultimately, may result in more effective disease control strategies to protect livestock.

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